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- (54) **SEQUENCING METHODS**
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- (57) **ABSTRACT**

The invention described herein solves challenges in providing a proficient, rapid and meaningful analysis of sequencing data. Methods and computer program products of the invention allow for a system to receive, analyze, and display sequencing data in real-time. The invention provides solutions to several difficulties encountered in assembling short sequencing-reads, and by doing so the invention improves the worth and significance of sequencing data.

23 Claims, 21 Drawing Sheets

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Co-pending U.S. Appl. No. 18/643,684, inventor Bava; Felice Alessio, filed Apr. 23, 2024.

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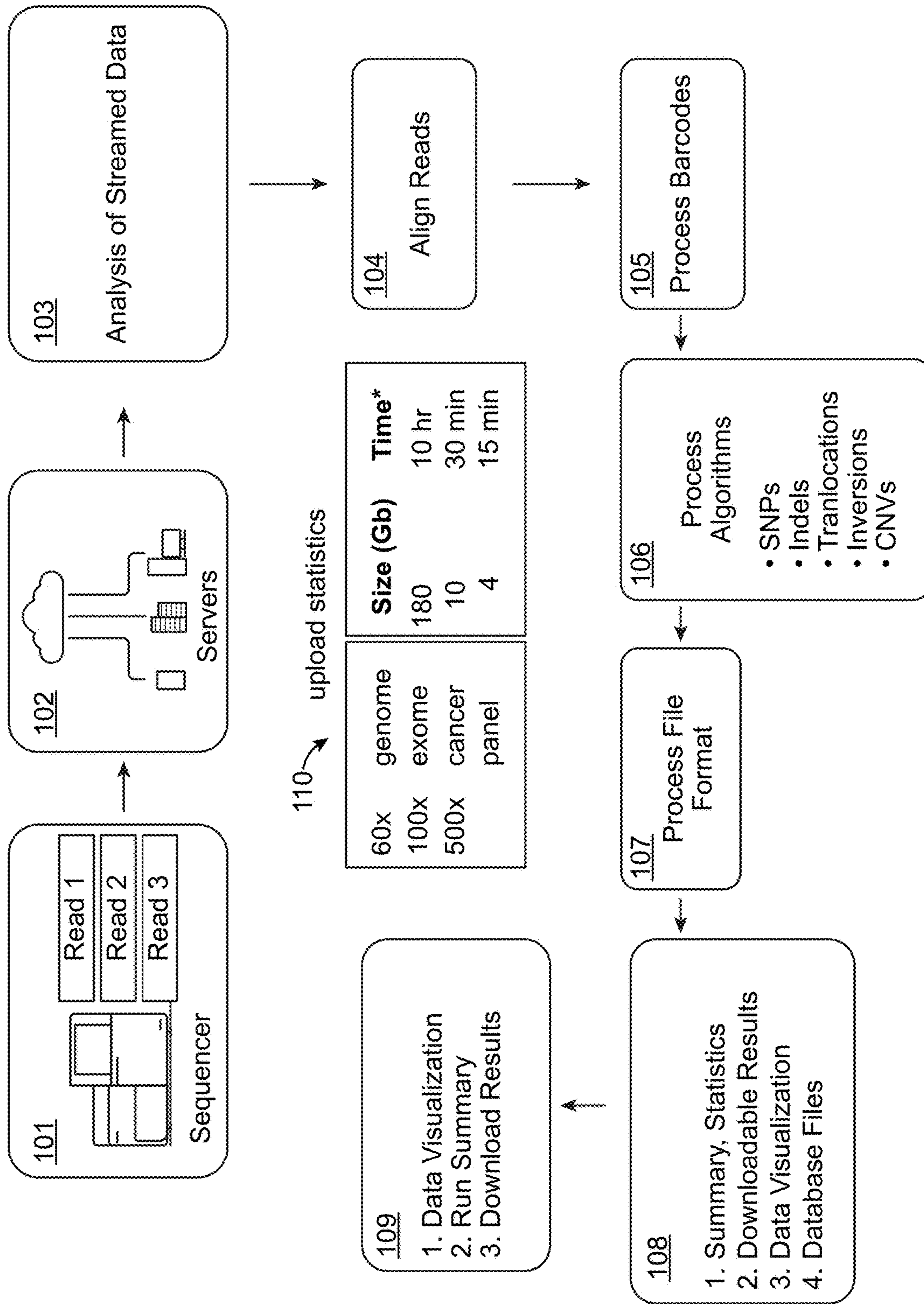


FIG. 1

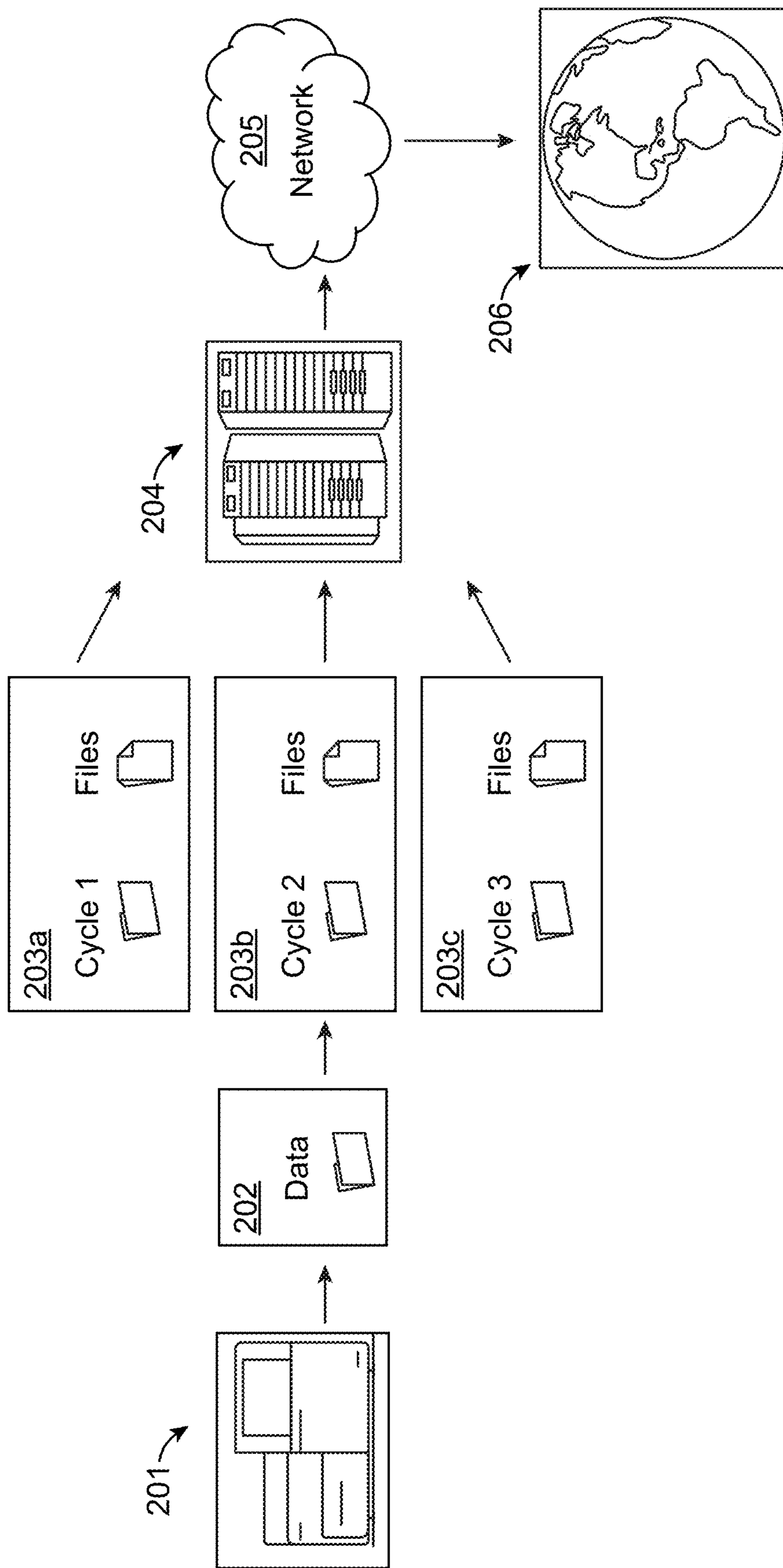


FIG. 2

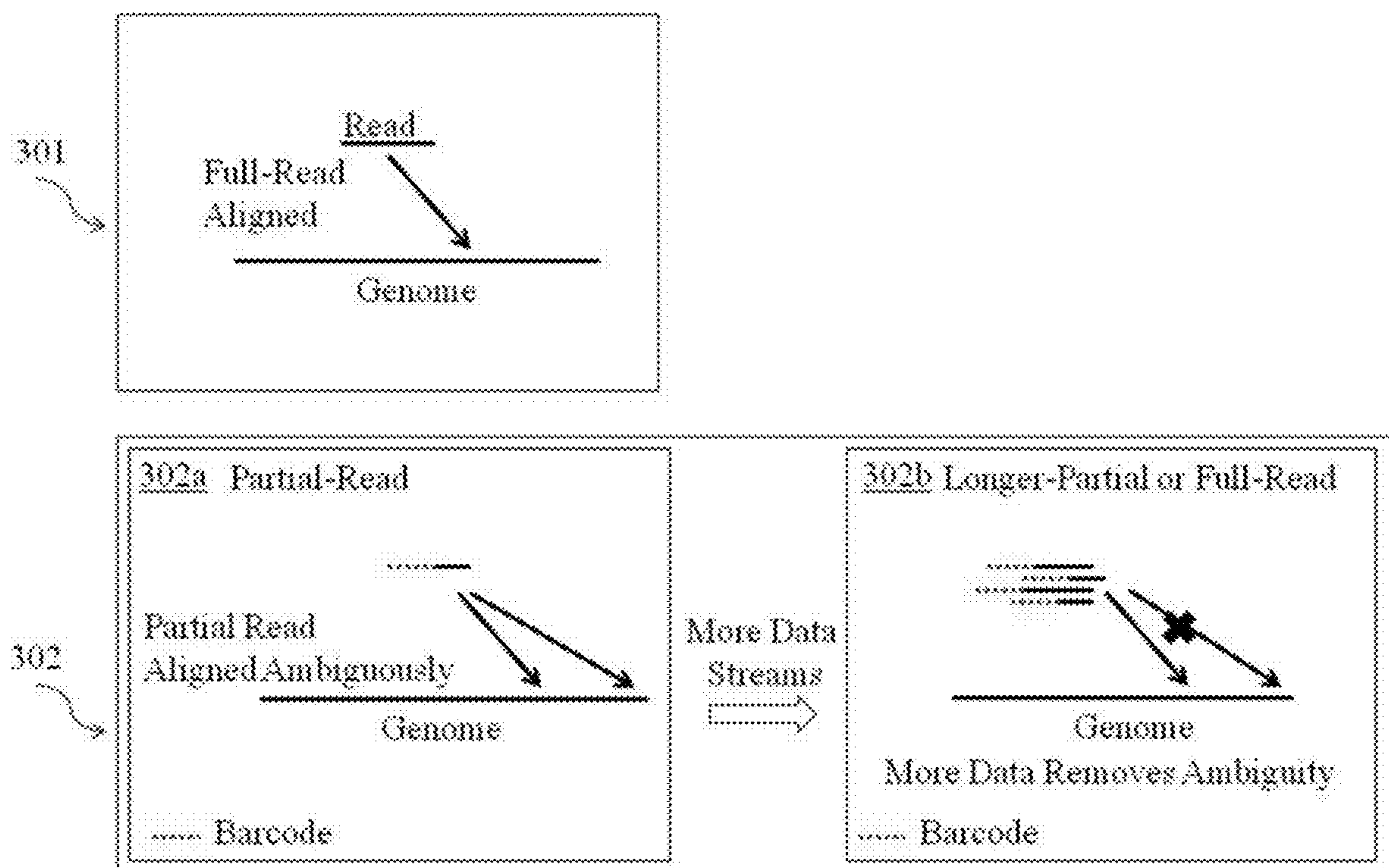


FIGURE 3

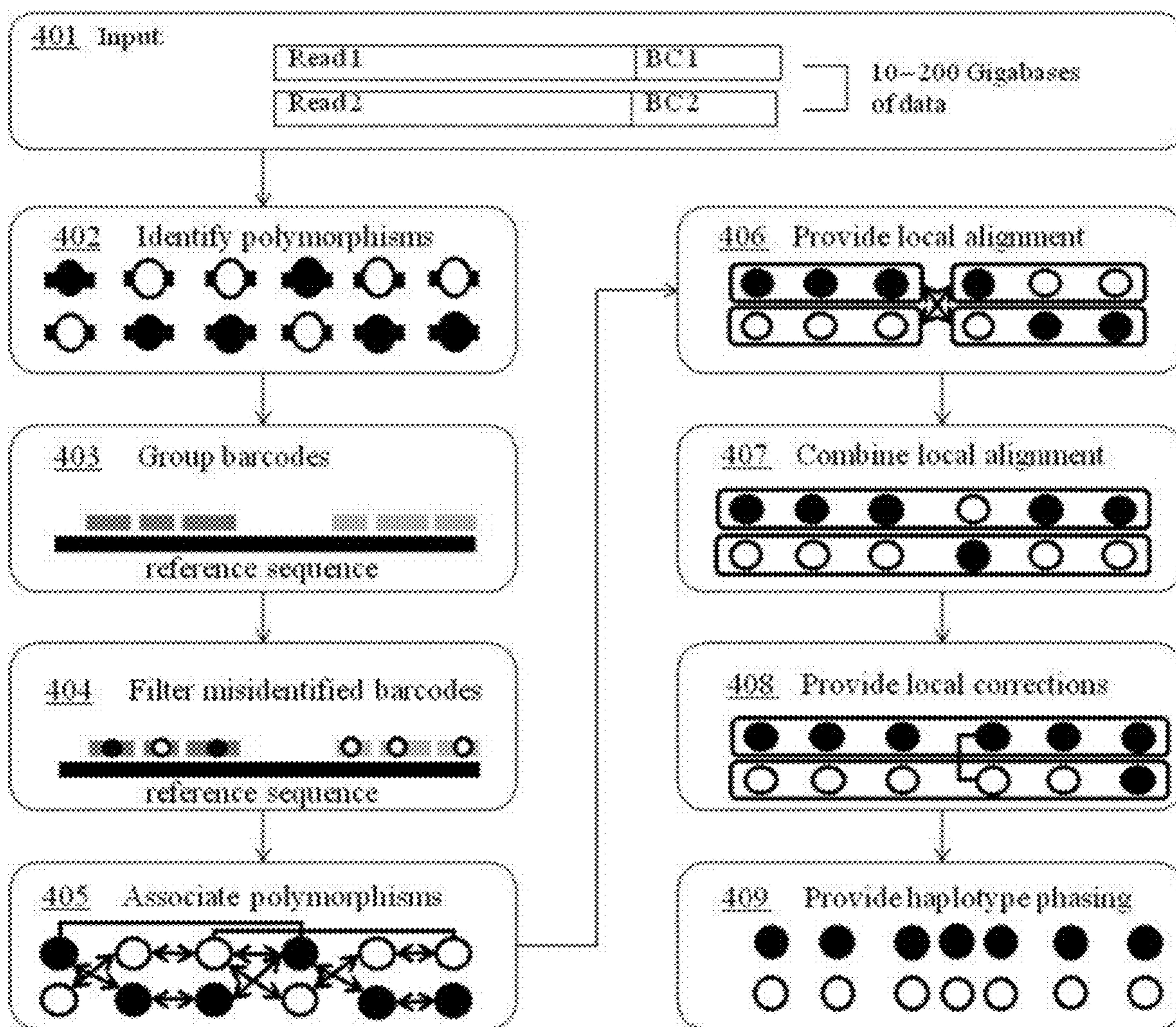


FIGURE 4

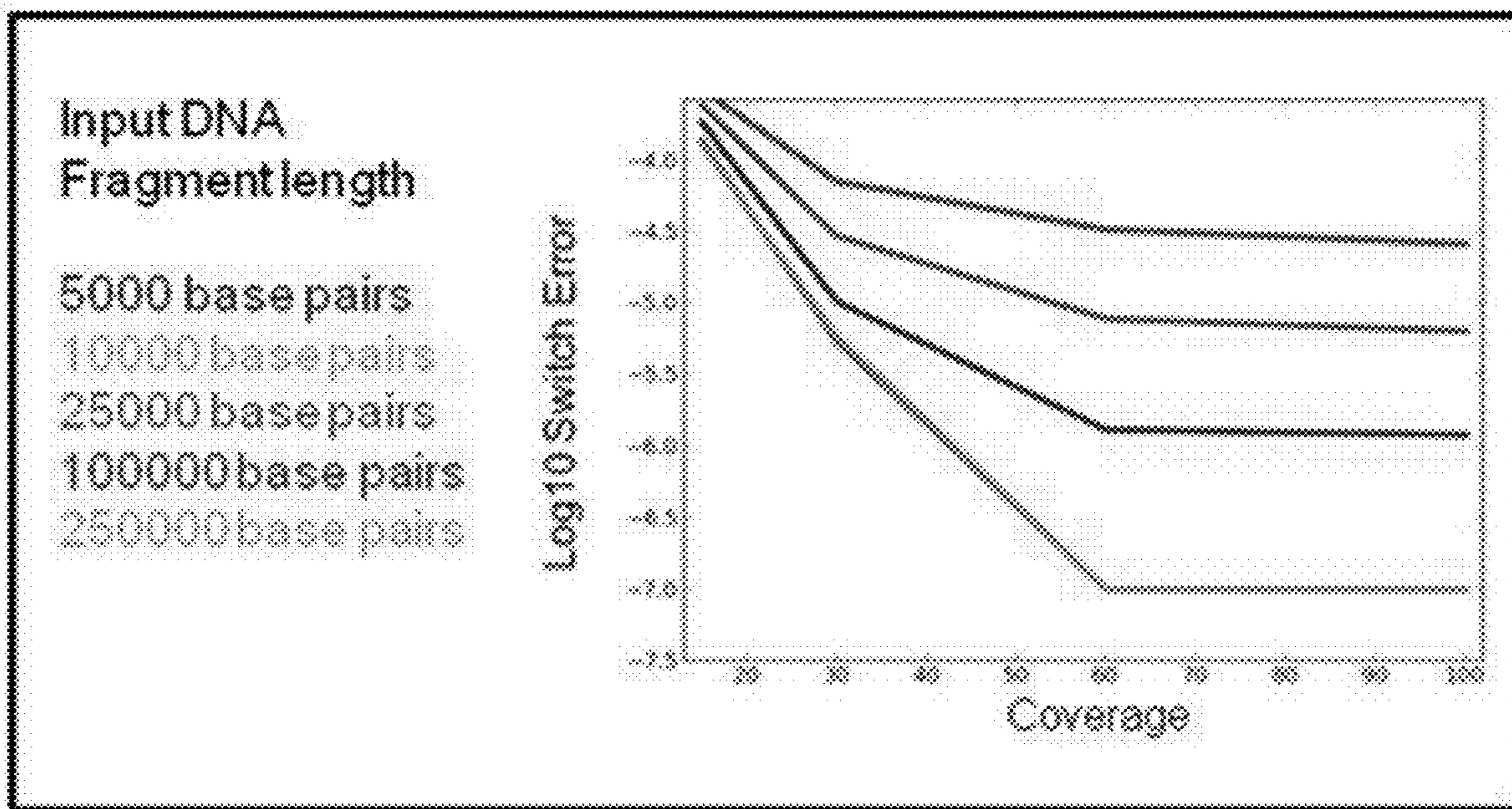


FIGURE 5

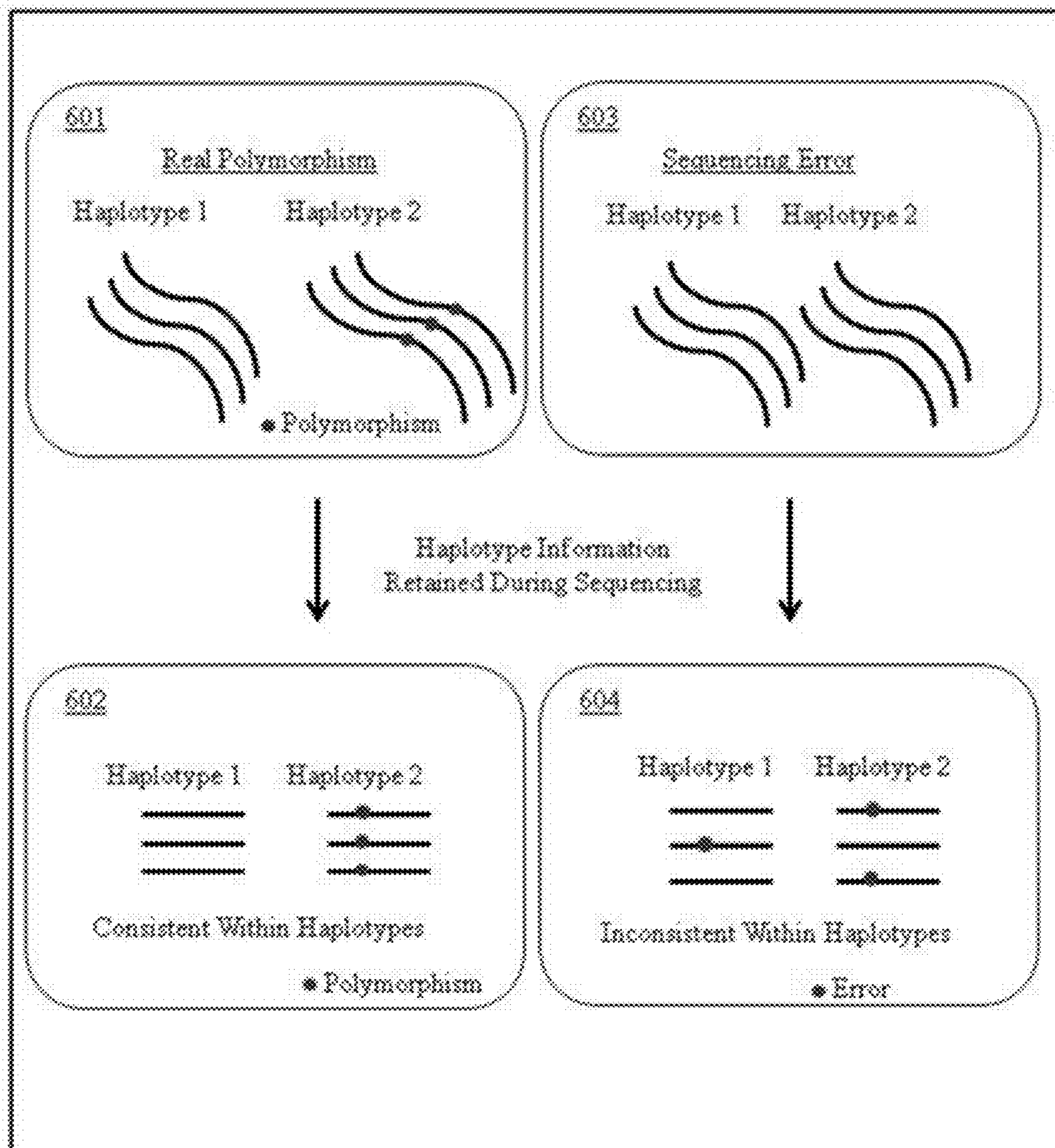


FIGURE 6

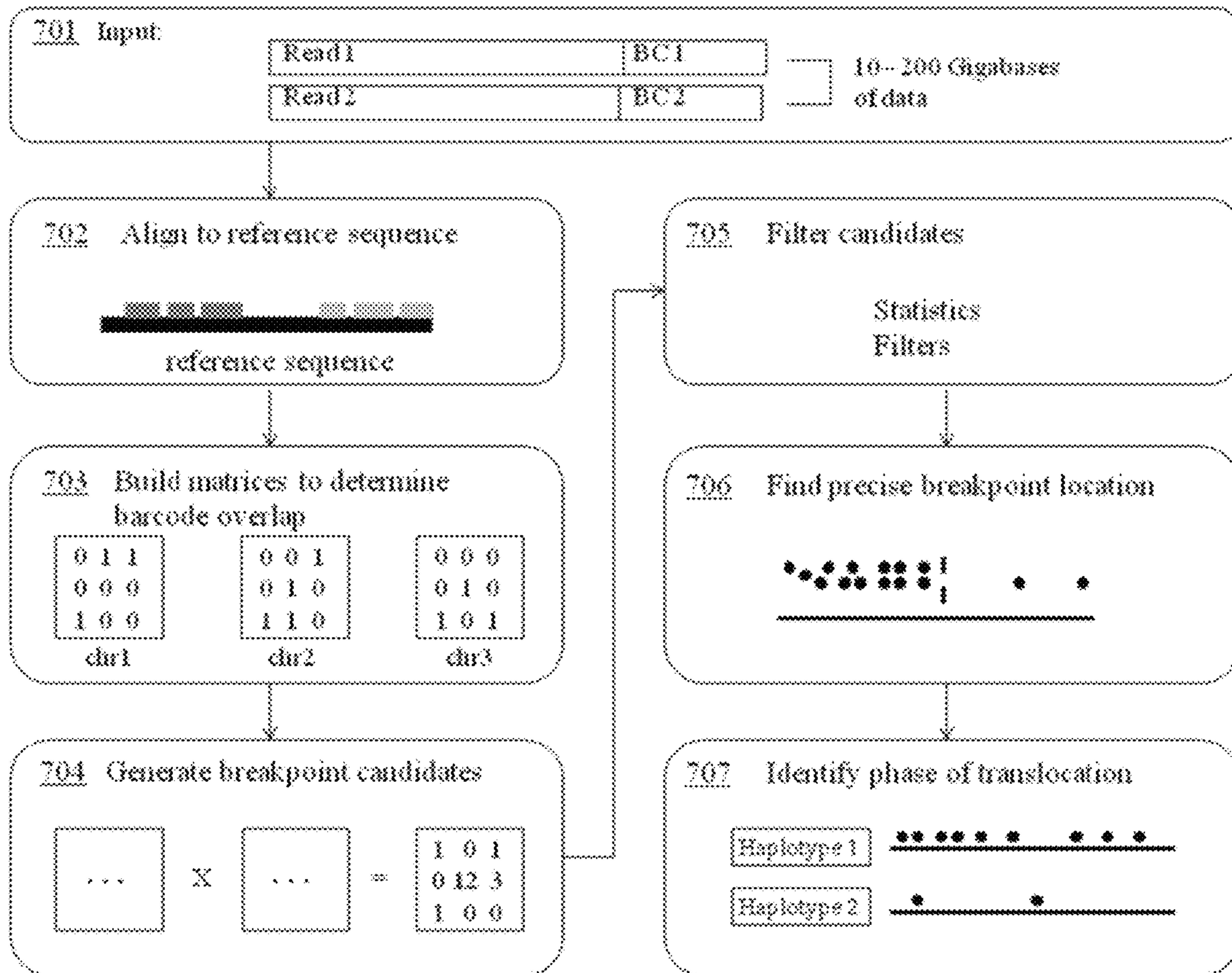


FIGURE 7

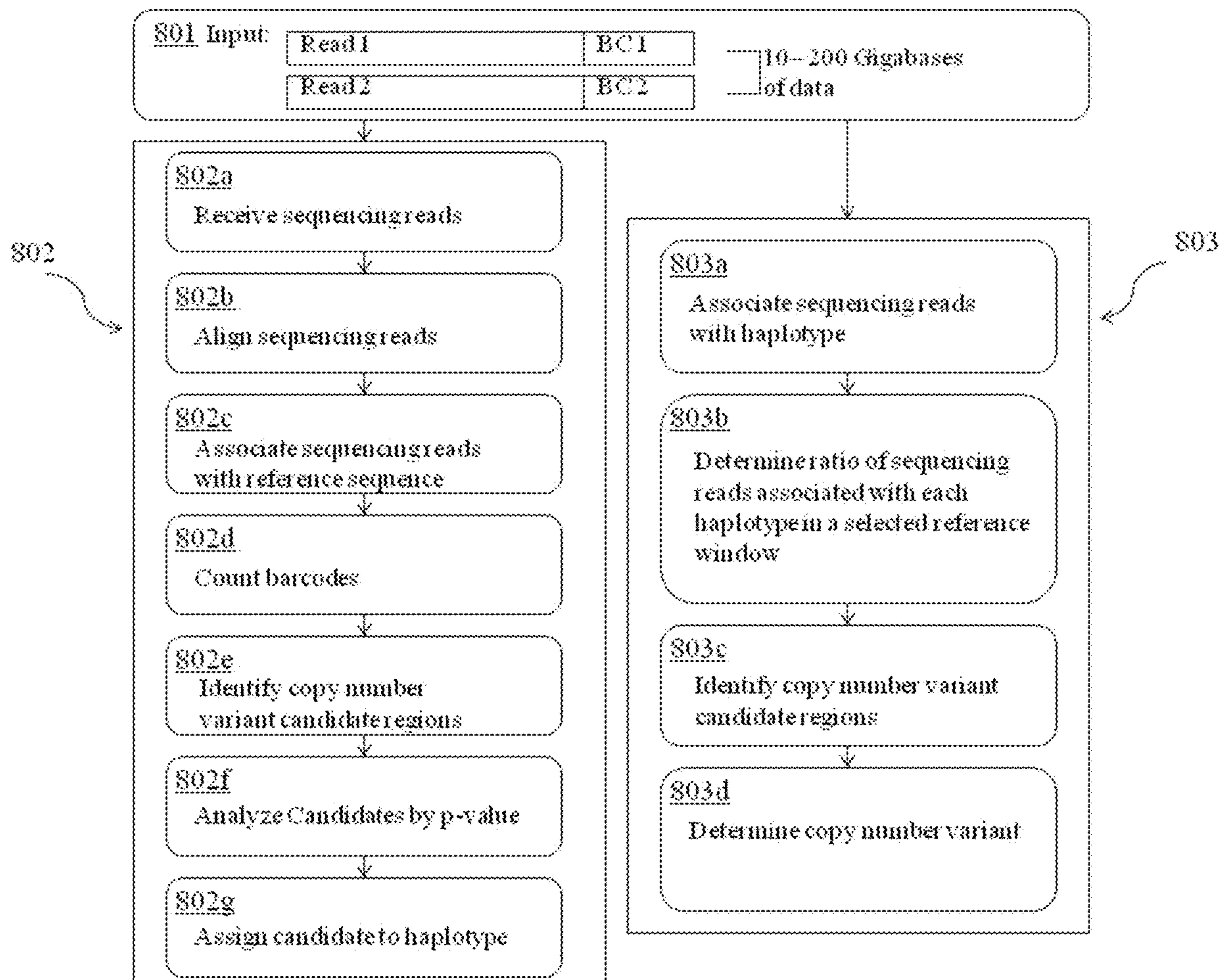


FIGURE 8

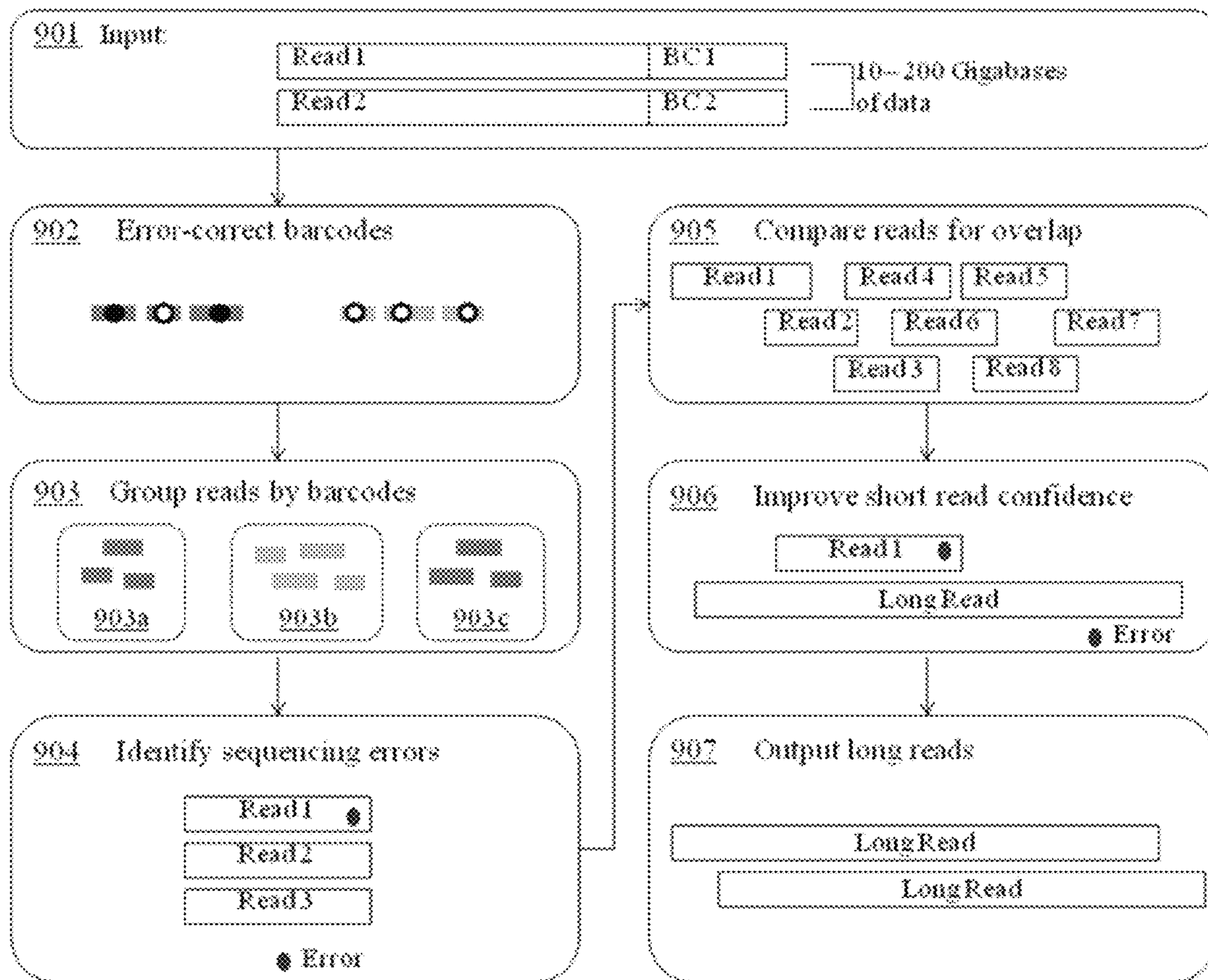


FIGURE 9

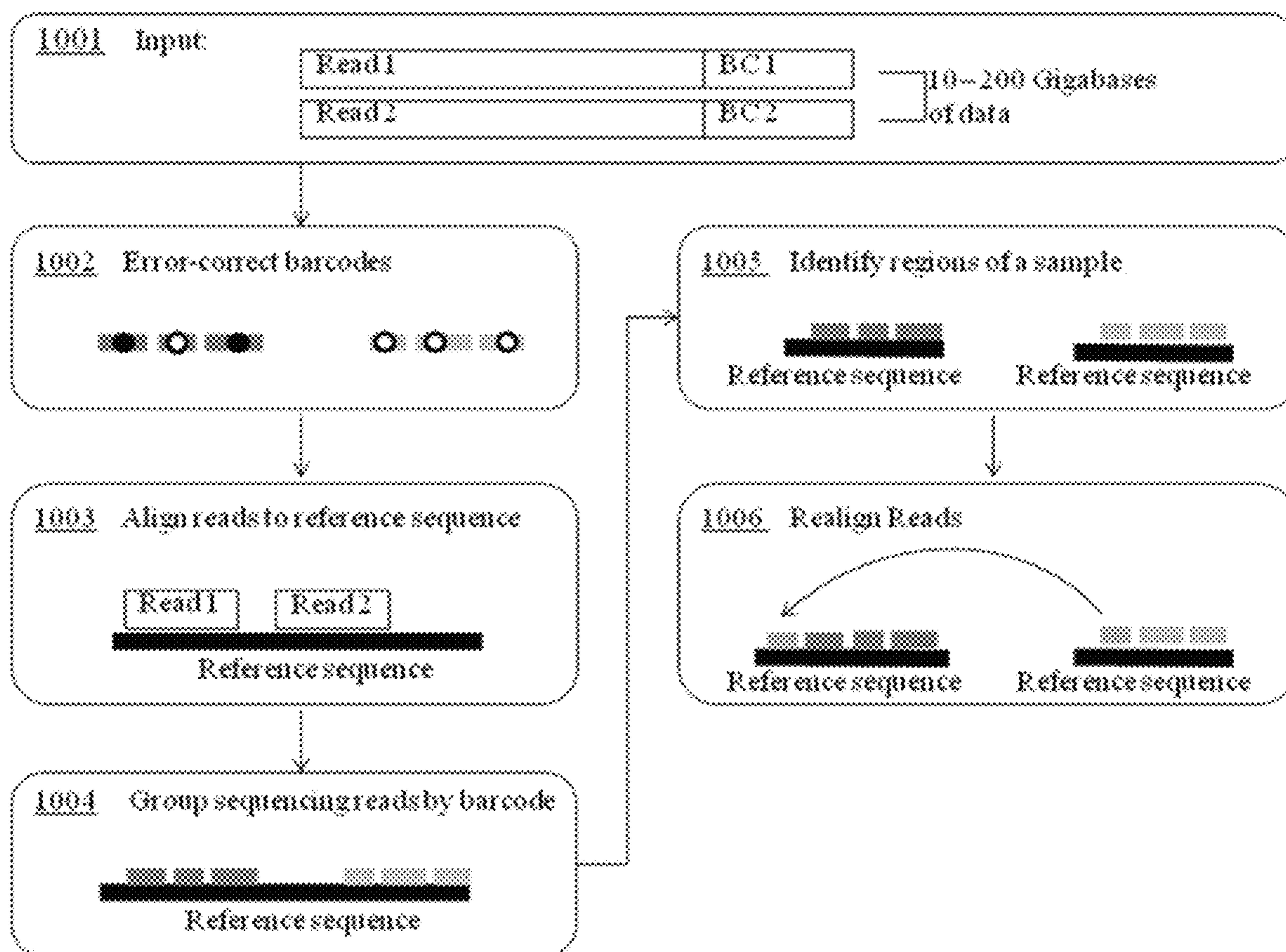


FIGURE 10

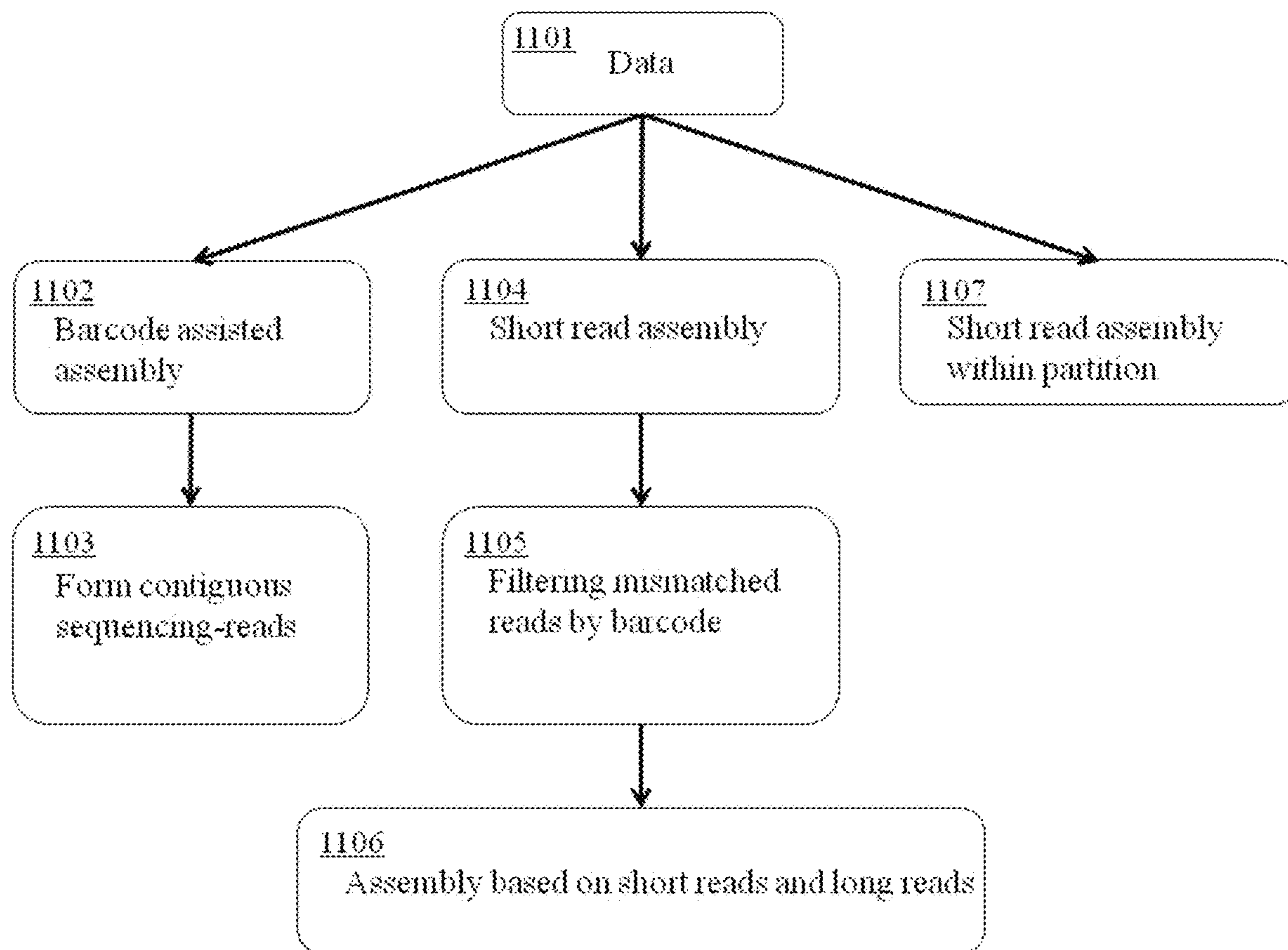


FIGURE 11

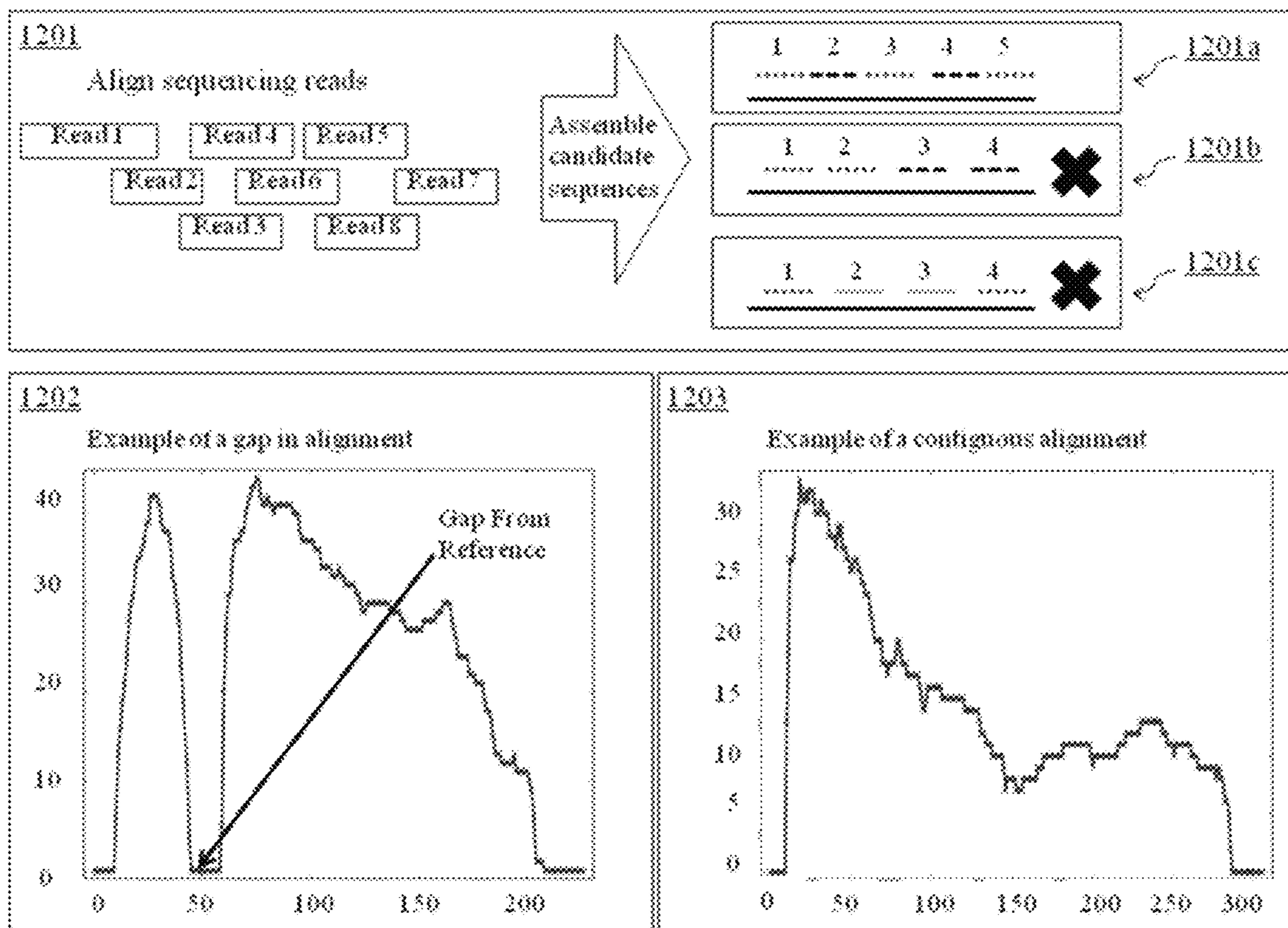


FIGURE 12

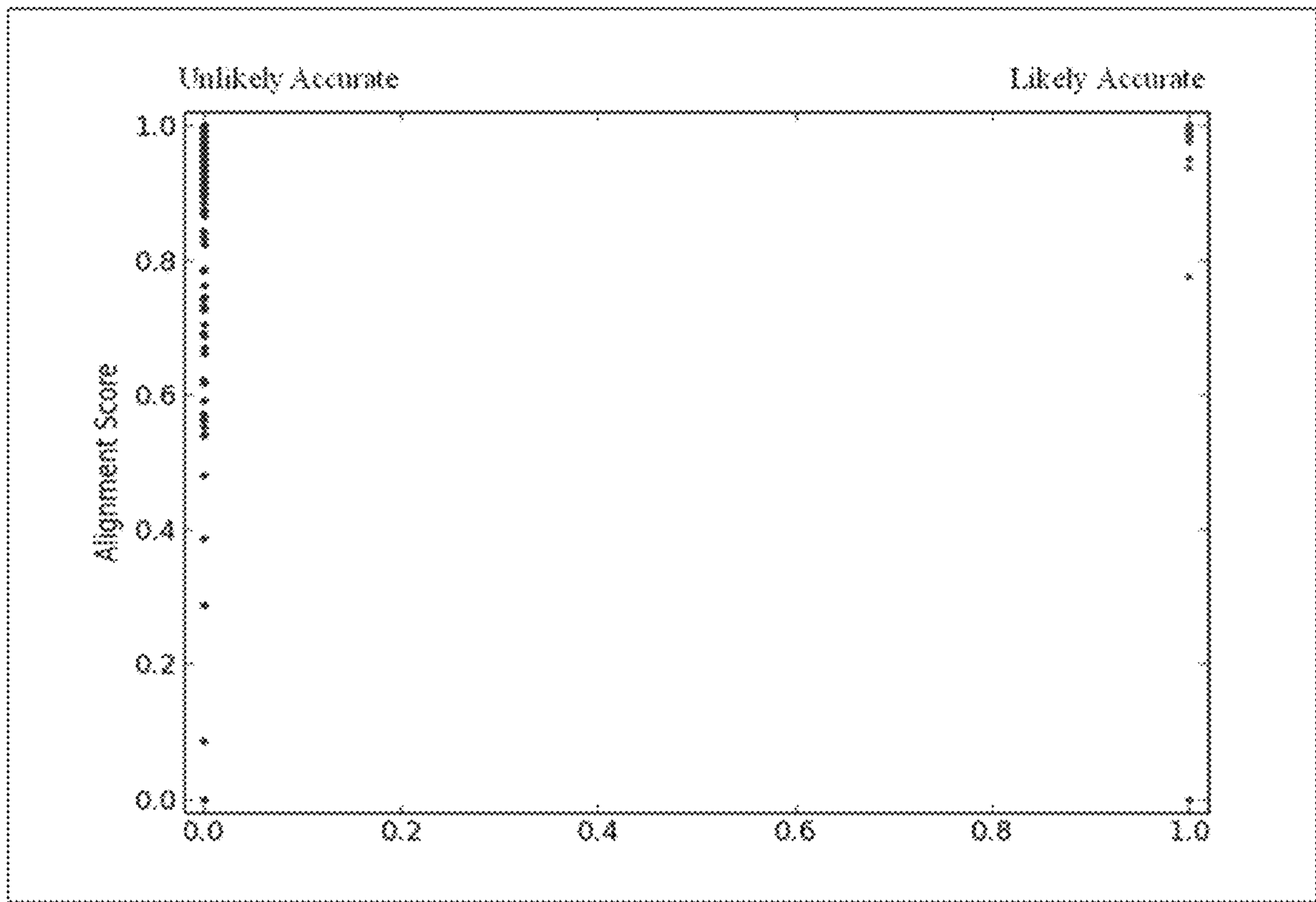


FIGURE 13

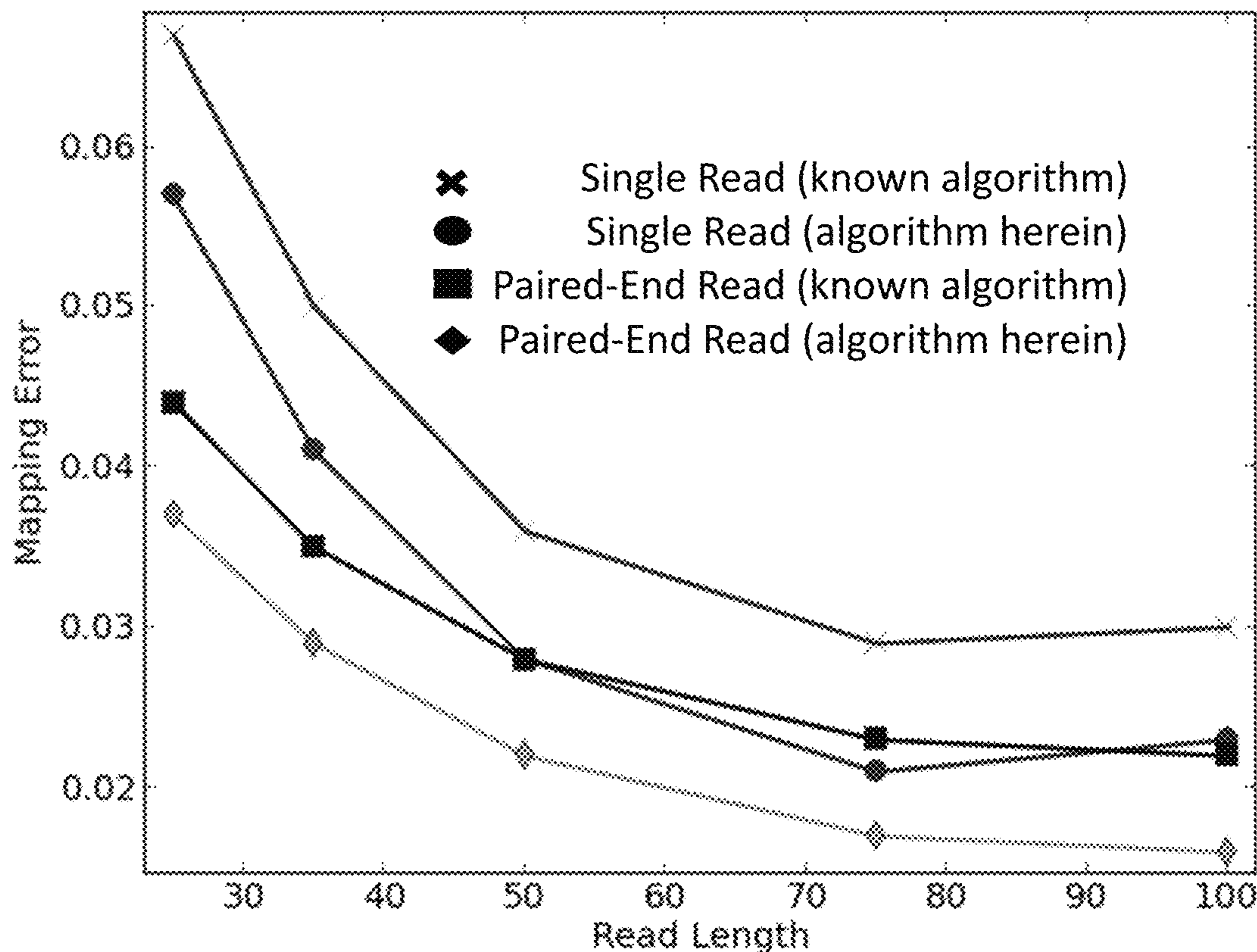


FIGURE 14

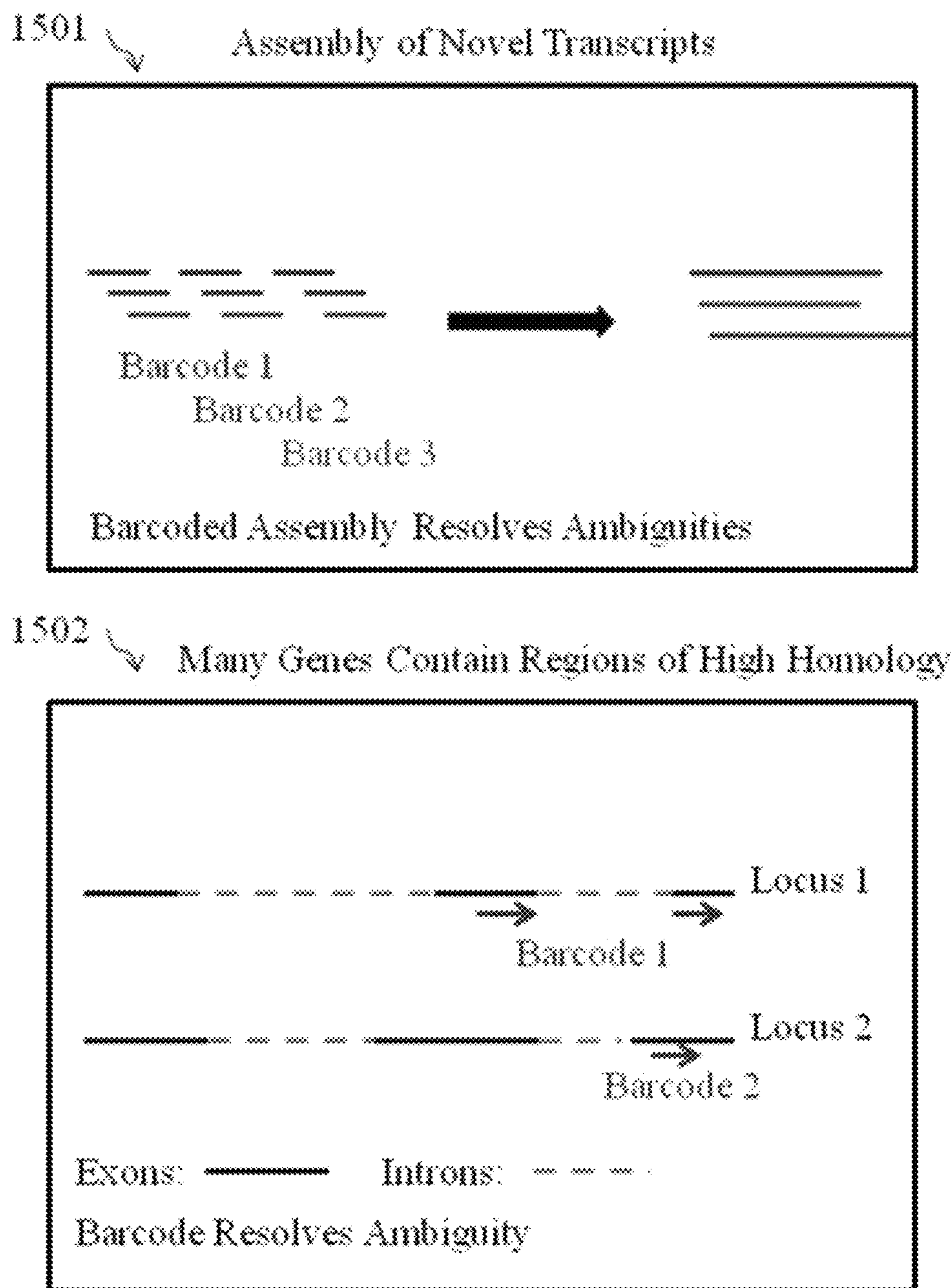


FIGURE 15

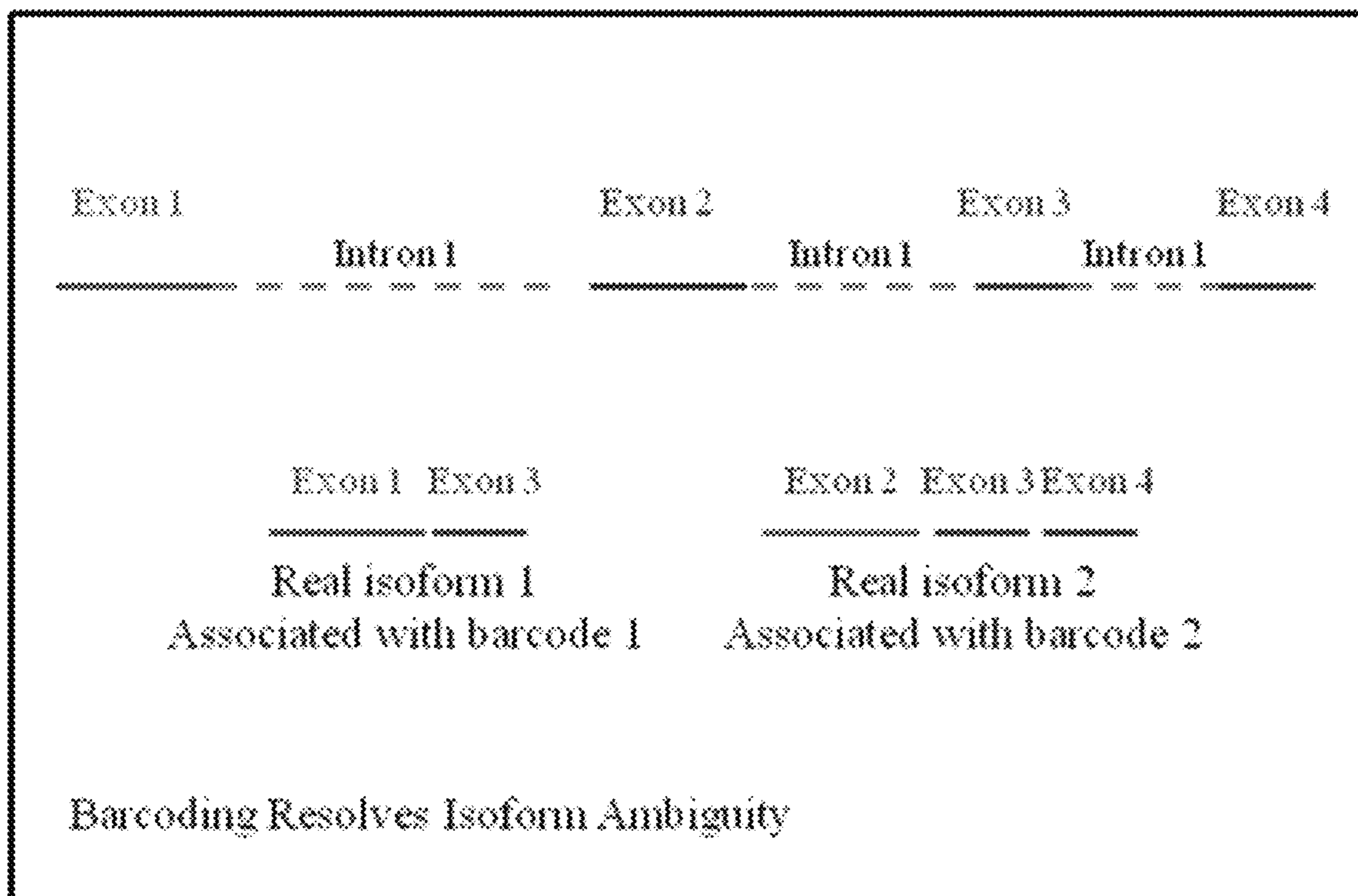


FIGURE 16

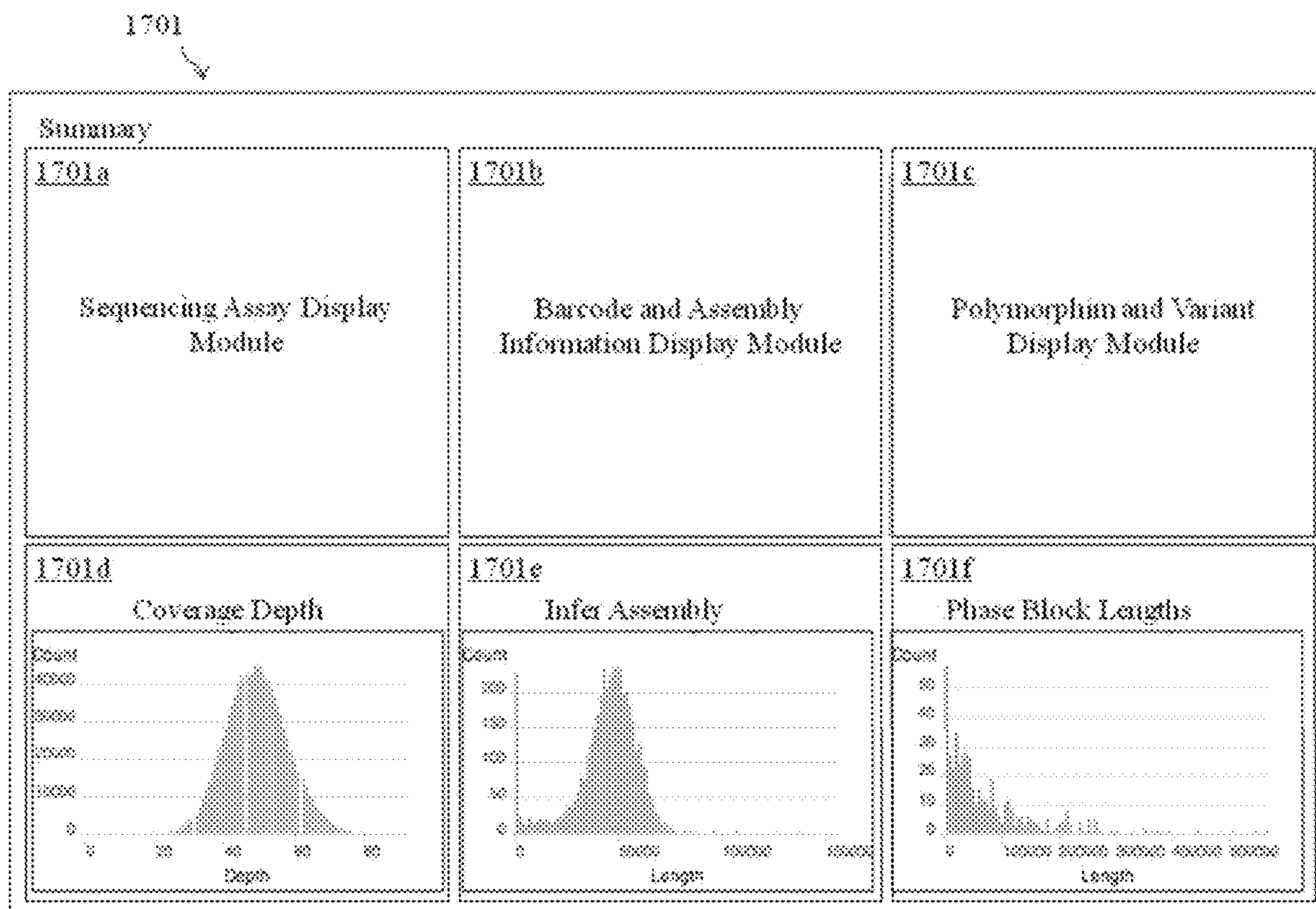


FIGURE 17

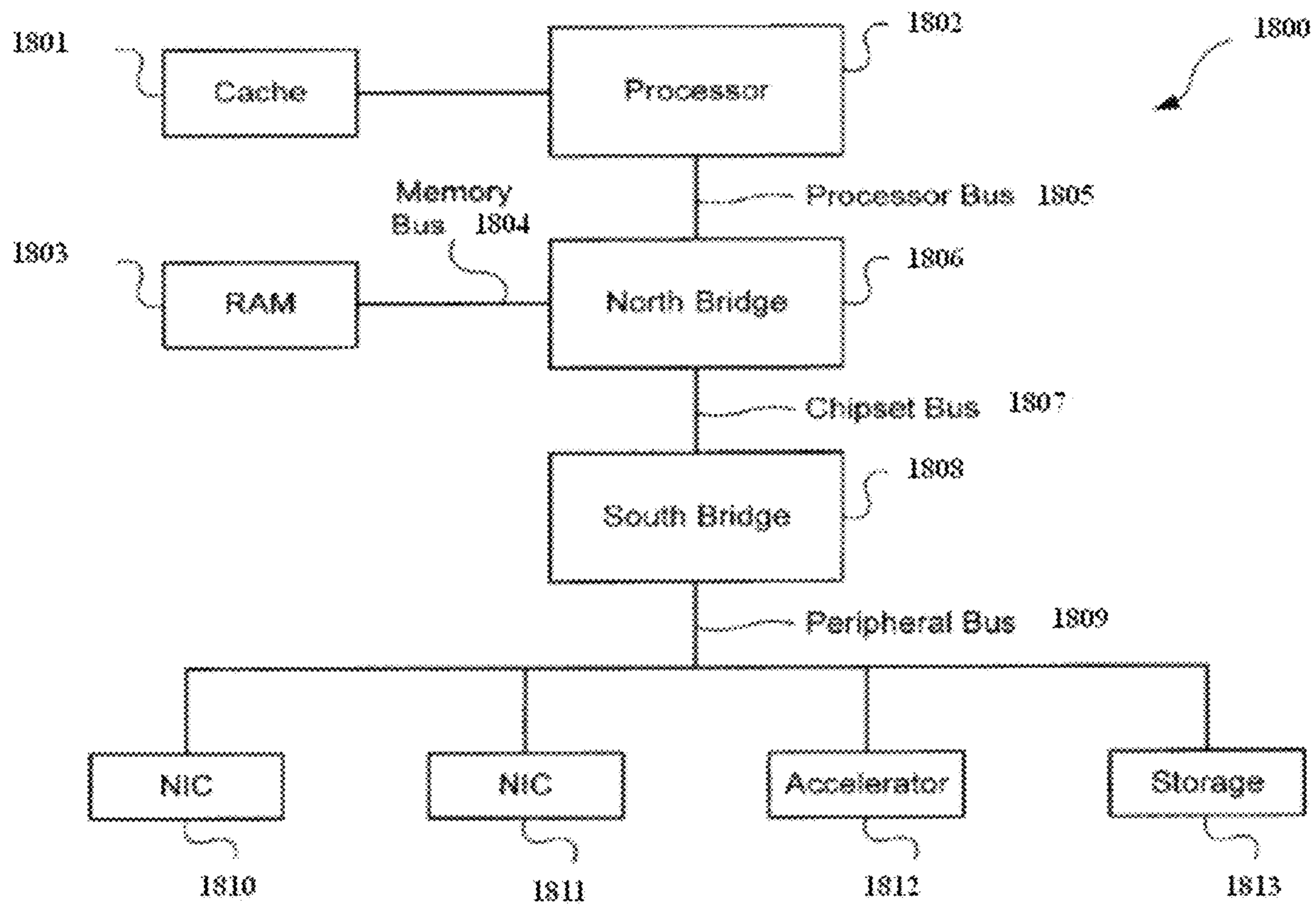


FIGURE 18

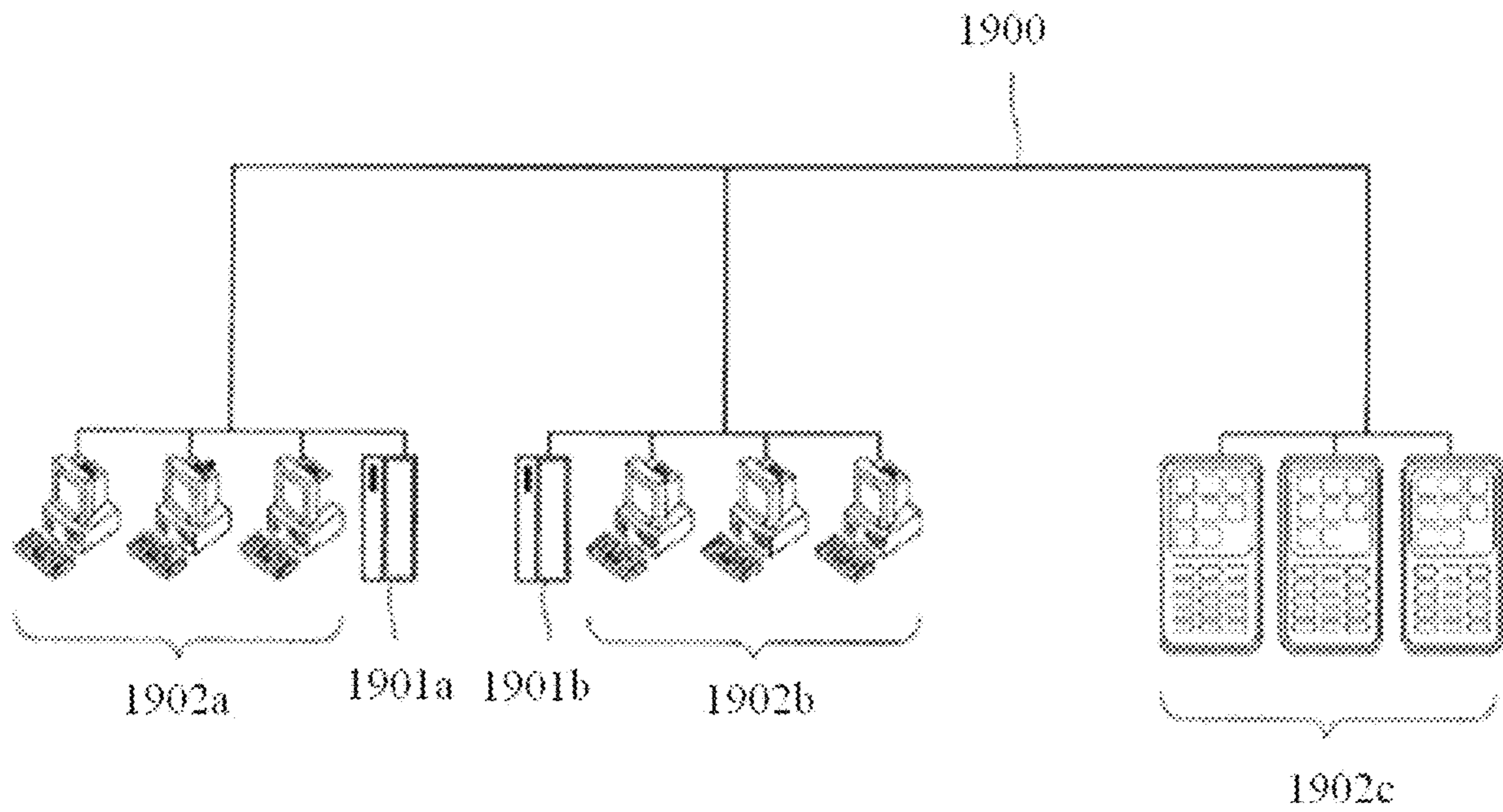


FIGURE 19

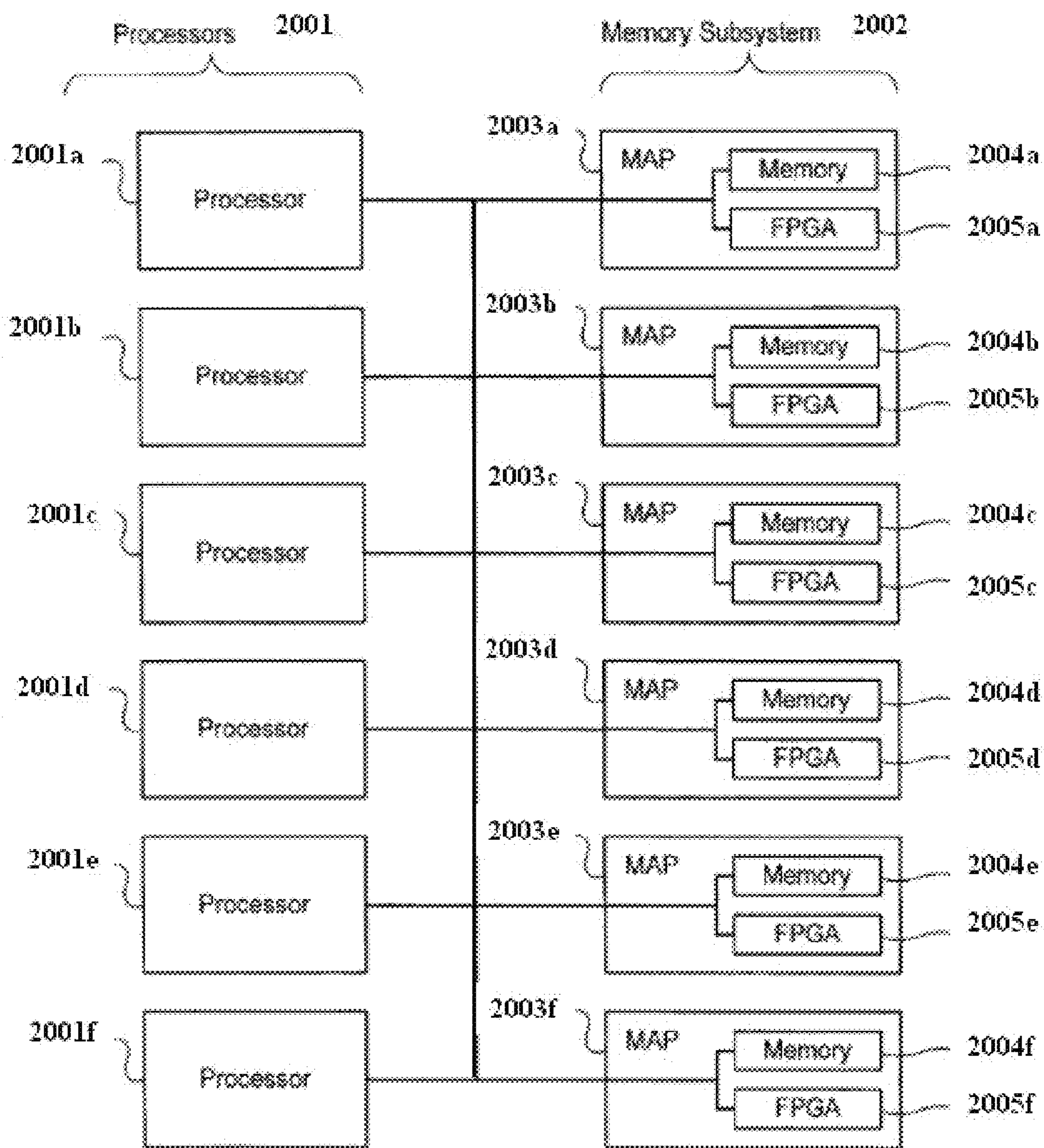


FIGURE 20

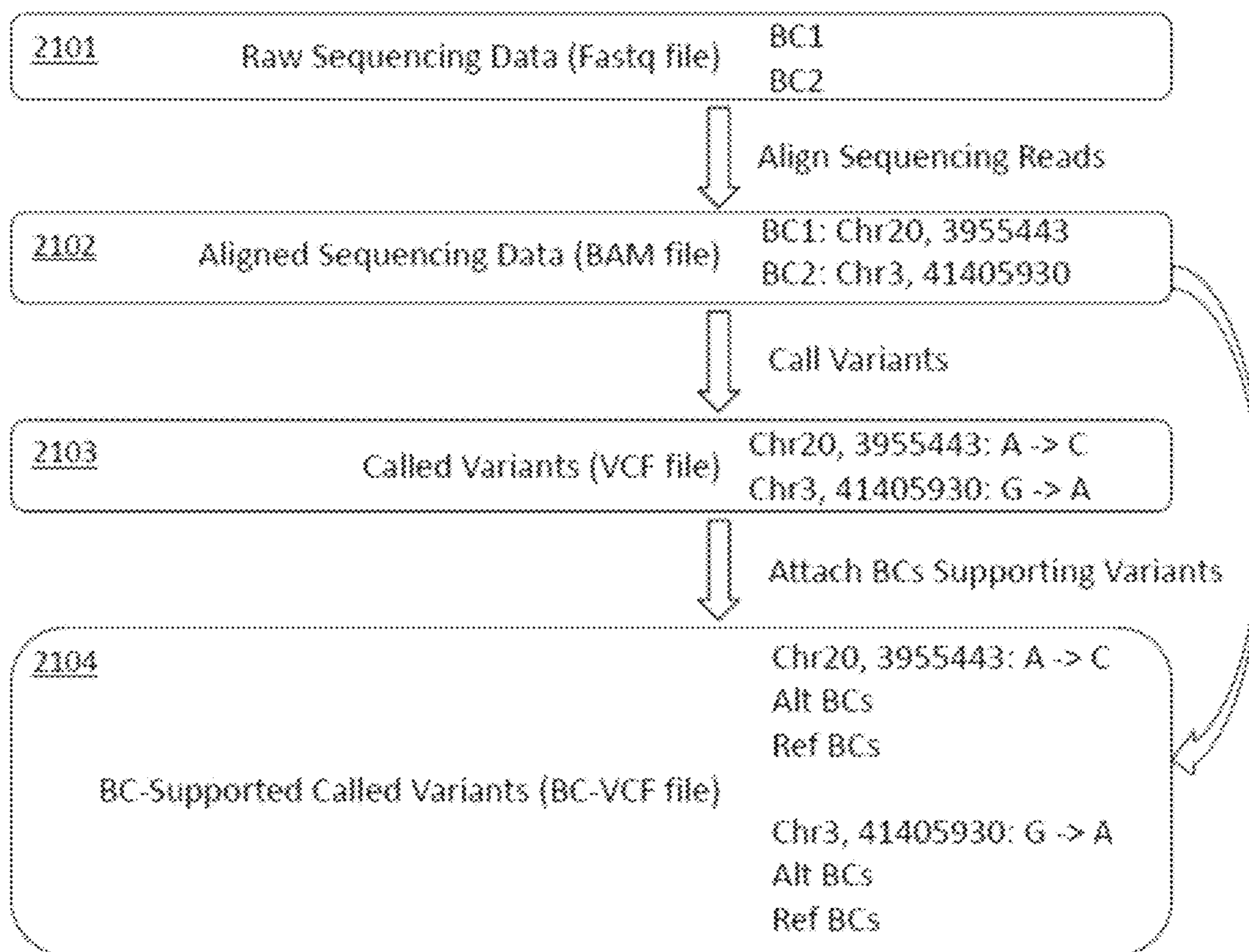


FIGURE 21

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SEQUENCING METHODS

CROSS REFERENCE

This application is a continuation of U.S. application Ser. No. 14/470,746, filed Aug. 27, 2014, now U.S. Pat. No. 10,395,758, which claims priority to U.S. Provisional Application No. 61/979,973, filed Apr. 15, 2014, U.S. Provisional Application No. 61/916,566, filed Dec. 16, 2013, and U.S. Provisional Application No. 61/872,597, filed Aug. 30, 2013, each of which is entirely incorporated herein by reference for all purposes.

BACKGROUND

The understanding of human genetics is rapidly expanding, fueled in part by developments in large-scale sequencing technologies. The results obtained from the sequencing of a genome, however, still present numerous production and bioinformatics challenges. The sheer volume of data obtained in a single sequencing experiment poses a remarkable data analysis challenge. The quality of the data, which often comprise millions to hundreds of millions of small sequence reads, poses yet another challenge. The development of streamlined, highly automated pipelines for data analysis is critical for transition from technology adoption to accelerated research and publication. Many obstacles remain in developing methods, algorithms, and computer program products for the analysis of sequencing data.

SUMMARY OF THE INVENTION

In some embodiments, the invention provides a method of analyzing sequencing data associated with a sample, the method comprising: a) receiving by a computer system a first sequencing read associated with a sequencing assay while the sequencing assay is in progress, wherein the computer system comprises a processor; and b) comparing by the processor the first sequencing read with another sequence to provide a comparison before the sequencing assay is complete.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module configured to receive sequencing data associated with a sequencing assay while the sequencing assay is in progress; and ii) an alignment module configured to align sequencing data while the sequencing assay is in progress; b) receiving by the receiving module a set of sequencing reads associated with the sequencing assay while the sequencing assay is in progress; and c) performing an alignment by the alignment module of at least one received sequencing read with another sequence while the sequencing assay is in progress.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is an overall schematic of a process wherein genomic data is streamed during sequencing and analyzed in real-time.

FIG. 2 is an overview of a process wherein genomic data can be shared within a global network.

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FIG. 3 illustrates example alignments of sequence reads to a reference genome.

FIG. 4 is a visual representation of example modules of a variant phasing algorithm that separates polymorphisms onto separate haplotypes.

FIG. 5 is a graphic representation of error frequency based on a fragment length of input DNA.

FIG. 6 illustrates a process that distinguishes a polymorphism from a sequencing error.

FIG. 7 is a graphic representation of an algorithm and example modules of a computer-program product that can identify a translocation.

FIG. 8 is a visual representation of example modules of a computer program product that can identify regions of a genome that have gained or lost copies.

FIG. 9 is a visual representation of example modules of a computer program product that can provide a digital representation of a sequence of a sample based on an assembly of short sequencing-reads into long sequencing reads.

FIG. 10 is a visual representation of example modules of a computer program product that identifies sequencing reads originated from the same starting piece of DNA.

FIG. 11 is a diagram illustrating multiple possible approaches to an assembly of sequencing reads.

FIG. 12 illustrates a method of filtering continuous sequences with barcodes.

FIG. 13 is a graphical representation of a plot of alignment scores.

FIG. 14 is an overview of a method utilizing barcodes to improve alignments.

FIG. 15 is an illustration of a barcode-assisted assembly of RNA transcripts.

FIG. 16 is an illustration of a barcode-assisted assembly of RNA transcripts applied to resolving ambiguities in assembling sequencing-reads of distinct RNA isoforms.

FIG. 17 illustrates a representative visual display of a data visualization/interface.

FIG. 18 is a block diagram illustrating a first example architecture of a computer system that can be used in connection with example embodiments of the present invention.

FIG. 19 is a diagram illustrating a computer network that can be used in connection with example embodiments of the present invention.

FIG. 20 is a block diagram illustrating a second example architecture of a computer system that can be used in connection with example embodiments of the present invention.

FIG. 21 illustrates a process for creating records in which variants identified in data analysis can be co-recorded with the barcodes used to identify the variant.

DETAILED DESCRIPTION

The disclosure provides methods and computer program products with applications in medical diagnosis and in the study of disease, health, population, and evolutionary genetics. The invention provides streaming, real-time processing of haplotype phasing, structural variant identification, variant correction, phasing of structural variants, de novo assembly, and barcoded alignment of sequencing reads. The invention can determine to which haplotype in a pair of haplotypes a sequencing read corresponds. A variant phasing algorithm of the invention can distinguish between alleles of maternal and of paternal origin. The invention presented herein can, for example, utilize a barcode present in a

sequencing read to distinguish identified polymorphisms onto separate haplotypes and reduce error rates.

Rapid advances in DNA and RNA sequencing technologies have generated a wealth of genomic data. Such data can provide a powerful new tool permitting the identification and annotation of the genome of an organism. Sequencing data can identify and characterize: a) genes responsible for giving an organism unique characteristics; b) genes conserved among species; c) genes that illustrate evolutionary changes among organisms; d) genes associated with health/disease; e) genes that confer disease resistance to an organism; f) genes associated with agricultural and nutritional properties; and/or g) genes associated with natural physiological processes, such as aging.

Despite the growing availability of sequencing data, the ability to process the information found within the data rapidly, effectively, and accurately remains a challenge. Sequencing diploid genomes presents the challenge of distinguishing nearly identical sequencing reads based on paternal or maternal origin. The ambiguity associated with distinguishing highly similar sequencing reads derived from distinct chromosomes introduces the issue of "phasing," or determining within which of the two chromosomes a sequencing read variant is associated. Questions of phasing are more complex than questions of distinguishing non-homologous chromosomes.

Single gene and/or genomic duplications provide another obstacle in the analysis of genomic data. Multiple copies of a variant/mutation can be present in one or more chromosomes, and the identification of the correct copy number can improve the value of genomic sequencing. Small insertions and/or deletions ("INDELs") can be found throughout a genome, the characterization of which is a challenge. The ability to characterize translocations and inversions, and corresponding breakpoints in a genome, can be valuable.

The invention provides an efficient solution to the greatest challenges in sequencing data analysis, and is capable of analyzing sequencing data that presented in distinct sequencing formats. The methods herein can distinguish variants on separate homologous chromosomes using phasing information. Phased sequencing can capture unique chromosomal content, including mutations that differ across chromosome copies, such as distinct mutations in distinct haplotypes, and variations within the same chromosome copy, while preserving haplotype phasing information. The computer program products and methods provided herein also distinguish between sequencing errors and real variations/mutations within a sequencing read with a low error rate, and identify and characterize, for example, INDELs, translocations, inversions, and multiple copies of a gene or chromosome.

Barcodes.

The term "barcode," as used herein, refers to a label, or identifier, that can be attached to an analyte to convey information about the analyte. Barcodes can have a variety of different formats, for example, barcodes can include: polynucleotide barcodes; random nucleic acid and/or amino acid sequences; and synthetic nucleic acid and/or amino acid sequences. A barcode can be attached to an analyte in a reversible or irreversible manner. A barcode can be added to, for example, a fragment of a DNA/RNA sample before, during, and/or after sequencing of the sample. Barcodes can allow for identification and/or quantification of individual sequencing-reads in real time.

Each partial-read, longer-partial, or full-read fragment generated during sequencing is associated with a barcode. In some embodiments, a sample can be associated with a

plurality of distinct barcodes. As more data streams from a sequencer to one or a plurality of servers, the sequencing reads can be processed based on barcodes, thereby grouping partial-reads, longer-partial, and full-reads associated with a common barcode. Barcode analysis resolves ambiguity and provides haplotype phasing information.

Some embodiments, for example polynucleotide sequencing, can utilize unique barcodes to identify a sequencing read and, for example, to assemble a larger digital representation of a sample from shorter sequencing reads. Depending upon the specific application, barcodes can be added to a sample before or during a sequencing of the sample. For example, a sample can be fragmented and physically separated prior to sequencing. A barcode can be added to a fragmented sample, for example, by dividing the fragmented sample into partitions so that one or more barcodes can be introduced into a particular partition. Each partition can contain a different set of barcodes. The presence of the same barcode on multiple sequences can provide information about the origin of the sequence. For example, a barcode can indicate that the sequence came from a particular partition and/or a specific region of a genome. In some embodiments, a first sequencing read and another sequence share a common barcode. This feature can be particularly useful for an assembly of a larger sequencing read. Depending upon the specific application, barcodes can be attached to analyte fragments in a reversible or irreversible manner. In some embodiments, an algorithm and a computer program product can detect, process, distinguish, group, separate, filter out, and assemble in real-time a plurality of streaming sequencing reads associated with a plurality of barcodes.

Streaming Sequencing Data.

The streaming sequencing algorithms and computer program products herein allow for the analysis of sequencing data to start even before a sequencing experiment is complete. The systems and methods of the invention can process partial information as the partial information is received. A sequencing read can comprise a barcode. In some embodiments, the invention described herein comprises a method of analyzing sequencing data associated with a sample, the method comprising: a) receiving by a computer system a first sequencing read associated with a sequencing assay while the sequencing experiment is in progress, wherein the computer system comprises a processor; and b) comparing by the processor the first sequencing read with another sequence to provide a comparison before the sequencing assay is complete. The other sequence can be another sequencing read or a reference sequence, such as a partial or complete reference genome. The other sequence can be obtained, for example, from an experiment or a database.

Any of the alignments, comparisons, analysis, and processes described herein can occur in real-time while a sequencing assay is still running and inputting data into the system. Data acquired initially or later in the processes are integrated seamlessly as if all the data had been received together. However, the rate at which the streaming methods can process data is much faster than that of a system that cannot stream and process data in real-time.

FIG. 1 illustrates an overall schematic of a process wherein genomic data are streamed during sequencing and analyzed in real-time with methods and computer-program products of the invention. Sequencing reads are generated with a Sequencer 101. Data associated with the sequencing reads are streamed in real-time and uploaded on Servers 102, for example, Amazon EC2™ cloud computing servers. Real-time streaming can allow the transfer of data from

partial sequencing reads into a server. As the data are streamed into servers, data analysis by one or more exemplary algorithms starts **103**. A computer-program product of the invention permits the process of data analysis to commence even as the sequencing is ongoing. In some embodiments, data from a sequencer of partial, full, and/or complete sequencing reads is streamed from a sequencer to a server as the sequencer runs.

The data collected in the process of streaming data from a sequencer to a server as the sequencer is running can be analyzed by streaming sequencing algorithms of the invention. In some embodiments, a computer program product can process partial information as streamed in real-time. In some embodiments, a computer program product comprises specific modules to analyze sequencing read data comprising barcodes. A module of a computer program product can, for example, align **104** a plurality of partial and/or full sequencing reads to a reference genome. The computer program products and methods of the invention contemplate the analysis of a variety of file formats for data types.

A module of a computer program product of the invention can process a plurality of different file formats for distinct data types. Non-limiting examples of file formats and associated sources of data are listed in TABLE 2.

TABLE 2

File Format	Source of Data
SAM (.sam)	Sequence alignment data
BAM (.bam)	Sequence alignment data
VCF (.vcf)	Variant call format
SEG (.seg)	Segmented data
CBS (.cbs)	Segmented data
MUT (.mut)	Mutation data
LOH (.loh)	Loss of Heterozygosity data
GFF (.gff)	Genome annotation data
GFF3 (.gff3)	Genome annotation data
BED (.bed)	Genome annotation data
GCT (.gct)	Gene expression data
RES (.res)	Gene expression data
GCT (.mai.gct)	RNAi data
IGV (.igv)	Numeric data
TAB (.tab)	Numeric data
WIG (.wig)	Numeric data
CN (.cn)	Copy number data
SNP (.snp)	Single nucleotide polymorphism data
TDF (.tdf)	ChIP-Seq, RNA-Seq data
GISTIC (.gistic)	Amplification/deletion data
FASTA	Reference sequence (nucleic acid or protein)

The invention can process barcodes and BAM File **105**, to perform a method described herein. A BAM file (.bam) is the binary version of a SAM file. A SAM file (.sam) is a tab-delimited text file that contains sequence alignment data. A module can process barcodes and BAM Files **105**, thereby associating a plurality of partial-read sequences with a genomic region.

One or a plurality of modules can be specifically programmed to provide, for example, an accurate solution to problems of haplotype phasing, Single Nucleotide Polymorphism (SNPs) identification, small insertion and deletion (INDELs) analysis, translocation analysis, inversion analysis, and/or copy number variant analysis (CNV) **106**. The data generated in **106** can be processed, for example, in Variant Call Format (VCF) File(s) format **107**. A module of a computer-program product of the invention processes data generated in **106** into a VCF File or another useful format **107**. The VCF specified format of a text file can be processed by an algorithm comprised within a computer program product of the invention to store gene sequence variations of

a sequencing read. Other formats, for example, the General Feature Format (GFF), can be processed by an algorithm and a computer program product of the invention to store all of the genetic data, including redundant sequences across the genome. In some embodiments, the selection of a file format that differentiates between redundant and non-redundant regions of a genome contributes to the efficiency of an analysis of sequencing reads.

A module of a computer program product can be programmed to produce files **108** for front-end analysis. A module **108** can, for example, produce and archive files corresponding to a summary and statistical analysis of the data associated with **101-107**. A module **108** can provide downloadable files, visualization files, and database files. All data associated with **101-108** can be visually displayed. A computer program product module **109** can be programmed to output the data on a visual display, to run a summary of the data, and to upload, transmit, save, store, and/or copy the results. Data can be drawn, for example, from a genome, from an exome, or from a cancer panel **110**.

FIG. 2 is an overall representation of the real-time transmission of a stream of data from partial-read sequences. **201** represents an illustrative sequencer generating sequencing reads from a sample. Data **202** from one or several sequencing cycles, for example, **203a**, **203b**, and **203c** is transmitted into a server **204**. The server **204** can transmit data from **203a**, **203b**, and **203c** to a network **205**. The network **205** can be a wired network or a wireless network, such as the cloud. All data described in **102-109** and **202-205** can be transmitted from one location to a different location. In some embodiments, the data can be transmitted from one geographic boundary to a second geographic boundary **206**. In some embodiments, the methods and computer program products of the invention can provide a tangible product, such as a report characterizing the sequencing data of a subject.

Alignment.

A sequence alignment can be a way of arranging nucleic acid or amino acid sequences to identify regions of similarity or overlap. An alignment of sequencing reads to a reference sequence can arrange the sequencing reads for construction of a candidate sequence. An alignment of a sequencing read to a reference sequence can be a global-alignment or a local-alignment. A global alignment can align a sequencing read to an entire length of a reference sequence. A local alignment can align a sequencing read or portions of a sequencing read to a window of a reference sequence. A local alignment can identify regions of similarity within sequencing reads and that can be widely divergent overall. An alignment can use an individual sequencing read or a candidate sequence derived from multiple sequencing reads.

FIG. 3 illustrates alignment of sequencing reads to a reference sequence. **301** illustrates an alignment of a full-read sequence to a reference genome. **302** illustrates reducing an ambiguity of an alignment (**302a**) of a partial-read sequence to a reference sequence as more data associated with longer-partial or full-read sequences with the same barcode are streamed and aligned to the reference genome (**302b**).

Variant Phasing and Non-Variant Phasing Algorithms.

A method herein can sort sequencing reads into separate haplotypes based on mutations, such as single nucleotide polymorphisms (SNPs), or lack thereof, present in the sequencing reads. A mutation can be identified based on a comparison among sequencing reads or against a reference sequence. FIG. 4 is a visual representation of illustrative

modules that can be programmed within a variant phasing algorithm to separate sequencing reads into separate haplotypes based on identified mutations. **401** illustrates sequencing Read 1, which is associated with Barcode 1, and sequencing Read 2, which is associated with Barcode 2. **401** also illustrates that an input received can be associated with a quantity of digital data, for example, 10-200 gigabases of data. A first module **402** can identify single nucleotide polymorphisms by comparing sequencing reads with a reference sequence. The module **402** can scan a sequencing-read for previously-characterized polymorphisms and uncharacterized polymorphisms. Each dot in **402** represents a SNP on a sequencing read, represented by a bar.

A module of a computer program product **403** can group sequencing reads sharing common barcodes for alignment against a reference sequence. A grouping of identical or similar barcodes can be aligned against a common region of the reference sequence to identify sequencing reads that originate from physically proximate regions of a sample. **403** illustrate two groups of sequencing reads represented by bars. The left triplet of reads share barcode and the right triplet of reads share the same barcode. Module **404** can filter out misidentified barcodes, for example, barcodes that are similar but not identical to those that had been previously grouped and barcodes associated with sequencing reads that do not contain an initially-identified single nucleotide polymorphism. **404** illustrates a sequencing read in the left triplet that mismatches those of the group, and is filtered out. Filtering out sequencing reads that do not comprise an initially-identified single nucleotide polymorphism can filter out a sequencing error.

Module **405** can associate and assemble sequencing reads comprising identical polymorphisms at the same position into a partial digital representation of a candidate sequence. **405** can associate sequencing reads with chromosome numbers, chromosomal position of the polymorphism, name of the polymorphism, and a p-value for the association of the sequencing reads. **405** can additionally provide a graphic representation of the association of sequencing reads comprising identical polymorphisms, for example, within a Manhattan plot, a Q-Q plot, or any other suitable graphical representation. A module **406** can provide a local alignment of **405** to a reference sequence.

Module **407** can combine local alignments into larger candidate sequences, including complete candidate chromosomes, as illustrated as the long horizontal bars in **407**. A statistical analysis can be used to determine which local alignments to combine. The analysis can involve determining the pattern of SNPs on each local alignment, and determining a statistical likelihood that different groups of SNPs exist on a common candidate sequence or chromosome. Non-limiting examples of sources of such statistical information include the number of barcodes shared across local alignments, the probabilities of sequencing errors on these barcodes, and the probabilities of sequencing errors on the reads used to infer SNPs. Module **408** can use similar statistical information or alignment data to suggest corrections to possible alignment errors made earlier in the process. **408** illustrates a correction being made by exchanging a sequencing read from one chromosome to the other based on the SNPs or lack thereof associated with those sequencing reads.

Module **409** can then identify that one of the candidate sequences corresponds to one haplotype or a diplotype, for example, the maternal haplotype, and the other candidate sequence corresponds to the other haplotype, such as the paternal haplotype. As illustrated in **409**, all SNPs or lack

thereof represented by dark dots are associated with one haplotype, and all SNPs or lack thereof associated with white dots are associated with the other haplotype. The system of FIG. **4** can distinguish one haplotype from the other based on the unique SNP patterns of the haplotypes, notwithstanding that the sequencing reads of one haplotype are otherwise identical to the sequencing reads of the other haplotype.

FIG. **5** is a graphic representation of improvements in the error rate of switching sequencing reads between haplotypes as the size of DNA fragment length increases. Coverage is the average number of reads representing a given nucleotide in an assembly digital sequence. High coverage can reduce the error rate of assigning nucleotides and sequencing reads to haplotypes correctly.

Polymorphisms and Sequencing Errors.

A method or a computer program product can distinguish single nucleotide polymorphisms from sequencing errors. FIG. **6** illustrates a process of variant correction utilizing haplotype phasing to distinguish a real mutation from sequencing errors. **601** represents a diploid sample wherein the haplotypes have already been distinguished by the process of FIG. **4**. Haplotype 1 does not contain a mutation, but haplotype 2 does contain a SNP. Each haplotype is represented by a triplet of sequencing reads that overlap at the site of the SNP. **602** illustrates that as the sequencing reads are processed into candidate sequences, the SNP associated with haplotype 2 sequencing reads is unambiguously assigned to haplotype 2, and haplotype 1 does not show a SNP at that position.

The diploid sample of **603** does not possess a SNP on either haplotype, but does suffer from an error in sequencing. Each haploid is represented by a triplet of sequencing reads, none of which shows a SNP. **604** illustrates the outcome obtained in the event of a sequencing error. The error appears among the sequencing reads at random, and is not unambiguously associated with one of the haplotypes. This random distribution of the erroneous nucleic acid is clearly distinguishable from the scenario of **602**, wherein the SNP is not distributed randomly among the sequencing reads of both haplotypes. Thus, when a putative SNP is identified in a sequencing assay, haploid phasing can verify the existence of a SNP instead of an error.

Translocations and Inversions.

Translocations and Inversions can lead to variability in the DNA, RNA, and protein constitution of a subject. Translocations have been identified as causes of cancers, infertility, and Down's syndrome. Translocations can occur naturally as reciprocal translocations between nonhomologous chromosomes. The invention provides computer program products and methods that identify previously known or unknown translocations and inversions within a set of sequencing reads of a sample.

In an analysis of a wild type genome, an individual barcode is ordinarily associated most prominently with one local region of the genome. Sequencing reads derived from physically-distant regions of a genome are less likely to share a common barcode. When barcoded sequencing reads are aligned to a reference genome, if remote portions of the reference genome are represented by sequencing reads that share a common barcode, then the possibility of a mutation, such as a translocation, can be investigated. A translocation in a sample would result in distant portions of a reference genome being proximate in a sample.

FIG. **7** illustrates an algorithm and modules of a computer program product that can determine a translocation, thereby identifying distant regions of a genome that had been

attached prior to translocation. **701** illustrates sequencing Read 1, which is associated with Barcode 1, and sequencing Read 2, which is associated with Barcode 2. **701** also illustrates the quantity of digital data associated with the sequencing reads, for example, 10-200 gigabases of data, which can be analyzed in real-time.

Module **702** aligns sequencing reads to a reference sequence, such as a reference chromosome or a reference genome, which can comprise multiple chromosomes. Each sequencing read independently comprises a barcode.

Module **703** represents barcodes distributed across three reference chromosomes (chr1, chr2, and chr3) in a reference genome. Each column of each matrix represents a region of each chromosome, and each row represents a specific barcode. The value at a point in a matrix indicates the number of times that the barcode was identified in the corresponding region of the chromosome. If regions have high numbers of barcodes in common, then those regions have a higher probability of being located close together in the sample, even if the regions appear far apart in the reference genome.

Module **704** processes matrices to identify candidate breakpoints. Matrices are multiplied, and the outcome can be scored to suggest a likelihood that a breakpoint exists on a certain reference chromosome in the reference genome. Module **705** then analyzes candidate breakpoints by p-value to determine which candidate breakpoints are most likely to be real.

Module **706** can identify the precise breakpoint in the reference genome leading to the translocation. Module **1006** aligns sequencing reads (dots) sharing a common barcode against a reference chromosome (horizontal bar) until a point is reached (vertical hash), beyond which sequencing reads possessing the common barcode no longer align with the reference chromosome. That point is the breakpoint of translocation. The breakpoint can be, for example, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or about 50 residues from a sequencing read that does align with the reference chromosome.

Module **707** can determine which haplotype in a pair of haplotypes is more likely to possess the translocation. Once the sequencing reads have been sorted by haplotype using the methods described herein, module **707** can determine which haplotype is more prominently associated with the translocation by determining which sequencing reads are associated with both the translocation and a particular haplotype.

Identifying Gained or Lost Copies.

The methods disclosed herein can identify gained or lost copies of a residue, sequence, or fragment, for example, a nucleotide residue or an amino acid residue. FIG. **8** illustrates modules of computer program products that can identify a copy number variant within a sample. **801** illustrates example sequencing Read 1, which is associated with Barcode 1, and example sequencing Read 2, which is associated with Barcode 2. **1101** also illustrates that an input received from a first sequencing-read and a second sequencing-read can be associated with a quantity of digital data, for example, 10-200 gigabases of data, which can be analyzed in real-time. **802** and **803** illustrate alternative approaches for identifying copy number variants within a sample.

A system **802** can receive a first sequencing read and a second sequencing read. The first sequencing read and the second sequencing read can each be independently associated with barcodes, which can be identical or distinct.

Module **802a** can receive a plurality of sequencing reads, which can total in the hundreds, thousands, tens of thousands, or greater. Module **802b** can align sequencing reads to a reference sequence and can further identify neighboring sequencing reads in the alignment, and determine whether the neighboring sequencing reads overlap at a non-barcode subsequence.

Module **802c** can associate a set of sequencing reads comprising a common barcode to a region (a "window") of a reference sequence. The system can receive an additional sequencing read that does not comprise the common barcode, which can be filtered out by module **802c**. Module **802d** can count a number of distinct barcodes that have been associated with a region of a reference sequence. Module **802e** can then identify candidate regions for a copy number variant based on the number of counted barcodes in a local region compared to global counts of the total number of barcodes in sample or in a region of the sample. If the number of barcode counts associated with a particular window is significantly greater than a statistical distribution, then the window can be designated as a candidate for a copy number mutation. The number of copies can be estimated based on the numbers of barcodes counted locally versus the numbers counted globally. Module **802f** can filter the identified regions based, for example, on a p-value to determine which candidate windows are most likely to contain a real mutation. Module **802g** can determine to which haplotype in a pair of haplotypes the copy number variant corresponds using a haplotype phasing algorithms provided herein.

In alternative **803**, the system begins the analysis using phasing information associated with received sequencing reads. Module **803a** phases a set of sequencing reads comprising barcodes by haplotype using methods described herein to provide two sets of sequencing reads: one associated with the first haplotype and the another associated with the second haplotype in a haplotype pair. Module **803b** determines or approximates a ratio of received sequencing reads associated with the first haplotype and received sequencing reads associated with the second haplotype within a window of a reference sequence. Module **803c** can identify candidate copy number variant regions based on a comparison of the number of barcodes within the window associated with one haplotype versus the other. For example, a ratio of barcodes associated with each haplotype that significantly deviates from a statistical expectation can suggest the presence of a copy number variant on a haplotype, and can provide an estimation of the number of copies. Module **803d** can filter the identified regions based, for example, on a p-value to determine which candidate windows are most likely to contain a real mutation.

A module can be programmed to execute any useful statistical calculation to determine, for example, the statistical significance of data suggesting a polymorphism, a copy number variant, a translocation, or an inversion, or the statistical likelihood of the existence of any of the foregoing. Non limiting example of a statistical analysis that can be performed include: a) analysis of variance (ANOVA); b) chi-squared test; c) factor analysis; d) Mann-Whitney U analysis; e) mean square weighted deviation (MSWD); f) Pearson product-moment correlation coefficient; g) regression analysis; h) Spearman's rank correlation coefficient; i) Student's t-test; j) time series analysis; and k) p-value analysis.

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Assembling Sequencing Reads.

A challenge in the analysis of sequencing data is to assemble short sequencing reads obtained experimentally into a longer digital representation of the sample unambiguously. Longer sequencing reads can provide faster and more reliable processing, a lower error rate, and a greater confidence level in alignments and construction of candidate sequences. FIG. 9 illustrates modules that can be coded within an assembly algorithm that assembles short sequencing reads into long sequencing reads. 901 illustrates sequencing Read 1, which is associated with Barcode 1, and sequencing Read 2, which is associated with Barcode 2. 901 also illustrates that an input received can be associated with a quantity of digital data, for example, 10-200 gigabases of data, which can be analyzed in real-time.

Module 902 identifies barcodes in received sequencing reads and searches for errors in the barcode reads. An error in reading a barcode ordinarily produces a sequence that deviates from a barcode only slightly, or has a high degree of homology with a real barcode, such as about 80%, about 85%, about 90%, about 95%, or about 97% homology with a real barcode. Errors in reading barcodes can also be identified by a count of the number of identical barcodes received. A particular barcode error should be received in much lower frequency than a real barcode. Thus, a barcode observed with a frequency that is statistically low and possesses high homology with another barcode that is received in much higher quantity is most likely an error in reading the other barcode.

Module 902 corrects the error in the erroneous barcode so the corresponding sequencing read can be processed correctly. 902 illustrates two triplets of sequencing reads. The right triplet shares a common barcode represented by a white dot. The left triplet shares a common barcode represented by a black dot; however, one sequencing read possesses the wrong barcode. 902 finds and corrects this error.

Module 903 then groups sequencing reads based on barcodes associated with the sequencing reads. 903 illustrates three groups, A, B, and C, of sequencing reads obtained from a source. All sequencing reads in a group share a barcode in common with the other sequencing reads in the same group, but do not share a barcode in common with any other group. For example, all sequencing reads in Group A are associated with barcode A exclusively, all sequencing reads in Group B are associated with barcode B exclusively, and all sequencing reads in Group C are associated with barcode C exclusively.

Once the sequencing reads have been grouped, module 904 identifies sequencing errors in the sequencing reads. Errors can be identified based on the frequency of certain subsequences occurring among sequencing reads of the same group. The confidence that a subsequence is correct increases as the number of sequencing reads containing the subsequence increases. However, a small number of sequencing reads can possess a subsequence that differs in a small number of residues from another subsequence that has been identified with a higher confidence level. The deviant subsequence can be identified as a sequence error and corrected to match the subsequence obtained at a higher confidence level.

Module 905 can compare short sequencing reads for overlapping subsequences. Multiple overlaps can be found, and the greatest statistical significance and confidence is assigned to long overlaps that are observed over the greatest number of sequencing reads. As high-confidence overlaps are found, the smaller sequencing reads can be assembled into increasingly larger sequencing reads.

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As the process continues, non-overlapping sequencing reads are eventually joined. For example, if a first sequencing read overlaps with a second sequencing read, but not with a third, and the second sequencing read does overlap with the third, module 905 can determine a candidate sequence organizing the first, second, and third sequencing reads into a single longer sequence.

Once longer sequencing reads have been generated, module 906 can improve data obtained from shorter sequencing reads by comparison of short sequencing reads with the longer sequencing reads. A short sequencing read can be aligned against a longer sequencing read that spans greater than the length of the short sequencing read. A high degree of homology in the alignment suggests with high confidence that both sequencing reads correspond to the same region of the sample. If the sequencing reads differ only slightly, such as in a single residue, module 906 can correct the short sequencing read to match the longer sequencing read because the longer sequencing read, being assembled from several barcoded sequencing reads, has a higher likelihood of being accurate. Module 907 can then output the long sequencing reads for use in development of candidate sequences.

Identifying Sequencing Reads Originating From a Common Source.

FIG. 10 is a visual representation of modules that can be encoded in an algorithm that identifies sequencing reads associated with a common biomolecule, such as a nucleic acid. 1001 illustrates sequencing Read 1, which is associated with Barcode 1, and sequencing Read 2, which is associated with Barcode 2. 1001 also illustrates that a sequencing read input received can be associated with a quantity of digital data, for example, 10-200 gigabases of data, which can be analyzed in real-time.

As sequencing reads are received and barcodes are recognized, module 1002 can correct errors in barcodes using methods described herein. The left triplet shown in 1002 illustrates a set of sequencing reads that have a mismatched barcode (white center). The right triplet shown in 1002 illustrates a set of sequencing reads that have properly matched barcodes. Module 1003 can then align sequencing reads to a reference, such as a reference genome. Module 1004 then groups sequencing reads within the alignment based on barcodes associated with each sequencing read, to provide groups of sequencing reads wherein each member of a group shares a common barcode. 1004 illustrates two such groups of barcoded sequencing reads aligned against the same reference genome, wherein both groups share the same common barcode.

Module 1005 can analyze the alignments to identify local regions of a sample. Overlapping sequencing reads sharing a common barcode that together provide a consensus region of a biomolecule represent a local region, and can be distinguished from another group of barcoded sequencing reads, which together provide a separate consensus region. As illustrated in 1004, both groups of sequencing reads share the same common barcode, and this commonality can suggest that both groups of sequencing reads represent local regions of a biomolecule that are spatially close. Very close regions can be physically contiguous in the sample.

Once close, or contiguous, regions have been identified, module 1006 can realign the sequencing reads to improve the quality of the alignment. When the local regions are contiguous, an individual sequencing read can potentially be aligned with one subregion that provides an alignment less accurate than that possible with another subregion. Dividing the barcoded group into distinguishable contiguous local

regions provides a second opportunity to reexamine the alignments, and when necessary, transfer a sequencing read from one subregion to another contiguous subregion to provide a more optimal alignment, as illustrated in **1006**. Assembling Short Sequencing Reads into a Digital Representation of a Sample.

Assembling short sequencing reads into longer contiguous and overlapping sequences suitable for generating candidate sequences is an expensive and time-consuming aspect of sequencing data interpretation. FIG. **11** provides an overview of multiple approaches to a high-level assembly of short sequencing reads into long sequencing reads, made more rapid and efficient by the processes provided herein. Sequencing data **1101** can comprise short sequencing reads associated with barcodes. One approach **1102** is to assemble short sequencing reads comprising identical barcodes into contiguous sequences, and to infer a consensus sequence of overlapping residues based on sequence overlaps. A second approach **1103** is to form a scaffold of contiguous sequencing reads based on an overlap of the sequencing reads and partial/global alignment to a reference genome. The short sequencing reads assembled in **1103** can comprise barcodes, and a barcode can be used as described herein to resolve alignment ambiguities. A third approach is to assemble short barcoded sequencing reads based on alignment **1104** of the short sequencing reads, filtering **1105** of mismatched sequencing reads based on the barcodes, and assembly **1106** of candidate sequences using both long reads and the filtered short reads. A fourth approach **1107** is identification of a group of short sequencing reads based on an understanding of the origin of the sequencing reads within a sequencer apparatus. Such an approach can efficiently assemble a set of overlapping short sequencing reads into longer sequences, and the short and long sequencing reads can be used together. Alternatively, process **1106** can be used directly without first aligning and filtering the short sequencing reads. All approaches are accessible via real-time processing.

FIG. **12** illustrates advantages of barcode-assisted assembly of a sequencing read. Pane **1201** illustrates the assembly of a candidate sequence from sequencing reads, and a selection of the best candidate sequence based on barcode analysis. **1201a** illustrates a candidate sequence derived from five sequencing reads, 1, 2, 3, 4, and 5, each of which corresponds to either barcode 1 (dashed) or barcode 2 (dotted). The pattern of barcodes 1 and 2 on the candidate sequence suggests that the sequencing reads associated with both barcodes originate from the same local region of the sample because both barcodes appear in overlapping series. Thus, the data obtained from the sequencing reads associated with barcode for barcode 2 mutually reinforce one another and add confidence to the candidate sequence of **1201a**.

1201b illustrates a candidate sequence derived from four sequencing-reads, 1, 2, 3, and 4, each of which corresponds to either barcode 1 or barcode 2. The alignment of **1201b** indicates that sequencing reads 1 and 2 are likely to correspond to proximate regions in a sample, and sequencing reads 3 and 4 are likely to correspond to proximate regions in a sample. However, since no overlap is observed between sequencing reads of differing barcodes, the data obtained from barcodes 1 and 2 do not reinforce one another. The analysis cannot ascertain unambiguously whether the alignment illustrated in **1201b** corresponds to a contiguous set of overlapping sequences or to remote regions of the sample.

1201c illustrates a candidate sequence derived from four sequencing reads, 1, 2, 3, and 4, each of which corresponds

to either barcode 1 or barcode 2. The alignment of **1201c** indicates that sequencing reads 3 and 4 are likely to correspond to proximate regions in a sample, but cannot provide an unambiguous relationship between sequencing reads 1 and 4. Since no overlap is observed between the alignments of **1201c** sequencing reads of differing barcodes, the data obtained from barcodes 1 and 2 do not reinforce one another. The analysis cannot ascertain unambiguously whether the alignment illustrated in **1201c** corresponds to a contiguous set of overlapping sequences or to remote regions of the sample. Of the three alignments, **1201a** offers the highest confidence option for constructing an accurate candidate sequence.

Panels **1202** and **1203** provide graphic representations of outputs of sequencing alignments against a reference sequence. Both graphs indicate the numbering of residues in the reference sequence on the x-axis, and, on the y-axis, the number of sequencing reads detected that correspond to the value of the x-axis.

1202 illustrates a negative spike around $x=50$, reaching down to $y=0$, over a span of residues of the reference sequence. This spike indicates a gap in the alignment between the sequencing reads and the reference sequence, a region of the reference sequence to which none of the sequencing reads correspond. The alignment producing this graph is unlikely to represent the sample accurately.

1203 illustrates a plot of a more accurate alignment. Each residue of the reference sequence is represented in multiple sequencing reads. The alignment producing this graph is more likely to represent the sample accurately.

FIG. **13** illustrates a plot scoring alignments generated by the methods described herein. A quality control module can generate a score for an alignment based on numerous factors such as percentage of reference residues represented and number of time represented, degree of sequencing read overlap, percentage of duplicates, number of errors, and numbers and ratios of barcodes detected. In FIG. **13**, an alignment producing the results of **1202** would be scored low, and would be grouped "Unlikely Accurate". An alignment producing the results of **1203** would be scored high, and would be grouped "Likely Accurate".

FIG. **14** illustrates simulated results of barcode-assisted alignments as a function of sequencing-read length and mapping error. Simulations were performed for both known sequencing analysis algorithms and for algorithms disclosed herein, using single barcode per read and paired-end barcodes per read systems. The plots indicate that in both cases of single or paired barcodes, the algorithms of the invention produce lower error rates at every read length investigated. RNA Sequencing Algorithms.

Alternative splicing events, which can be common in eukaryotic RNA, pose challenges to the identification of distinct splicing variants with sequencing technologies. The invention provides an effective and accurate method to resolve ambiguities present in sequencing reads generated with RNA sequencing technologies. Non-limiting examples of classes of RNAs that can be analyzed with a method herein include: messenger RNAs (mRNAs), transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs), small interfering RNAs (siRNAs), piwi-interacting (piRNAs), non-coding RNAs (ncRNAs), long non-coding RNAs, (lncRNAs), and fragments of any of the foregoing.

The methods herein of analyzing sequencing data solve challenges in analyzing RNA sequencing data associated with genes that are alternatively spliced. FIG. **15** illustrates a process wherein an association of a specific barcode with

individual transcripts unambiguously identifies each transcript, whether the transcript contains alternatively spliced exons or not.

In **1501**, each barcode is associated with a distinct transcript. For example, a translocation frequently identified in prostate cancer can include fusions of the Tmprss2 gene, and the ETS transcription factor gene ERG to generate Tmprss2-ERG translocations. The presence of an identical barcode in a transcript comprising a fragment of the Tmprss2 gene and a fragment of the ERG gene can designate the presence of a Tmprss2-ERG translocation in a sample.

1502 illustrates a method wherein an association of a barcode with a particular locus can indicate the locus of origin of a transcript. For example, a mutation within the first exon of the Adenomatous polyposis coli (APC) gene of a human subject can be associated with a 95% likelihood of the subject developing familial adenomatous polyposis (FAP). A method of the invention can associate an RNA transcript with a haplotype, thereby unambiguously identifying the entirety of the transcript comprising the mutation.

Another challenge presented by RNA sequencing analysis is the reconstruction of short sequencing reads into correct isoforms. FIG. **16** illustrates a barcode-assisted assembly of RNA transcripts applied to resolving ambiguities in assembling sequencing reads of distinct RNA isoforms. The association of identical barcodes to different RNA isoforms provides an effective method to resolve ambiguities in assembling short sequencing-reads generated in RNA sequencing.

Visual Output.

The methods and computer program products provided herein can output information associated with a sequencing assay in real-time as an assay runs. Output information can include sequencing parameters, data, sequencing reads, candidate sequences, alignments, comparisons, mutations, errors, reference sequences used, and information associated with the assay instrument, user, or client. A system can track assays occurring on multiple instruments simultaneously and identify the instruments being used by make, model, serial number, owner, or user. In doing so, the output module can provide information about the end users and their preferences and parameters. A visual output can track the construction of multiple candidate sequences simultaneously, and display multiple candidate chromosomes simultaneously for comparison.

A user of the output system can monitor progress of an analysis, and can interact with information during the analysis, either in static or real time, and upon conclusion of an analysis. The user can make notations in the interface, such as posting comments on an analysis and flagging points of interest, such as potential errors. The user can obtain information from the interface, including data, data processing results, and information describing an instrument, computer, client, facility, sample, or subject associated with the sequencing data.

FIG. **17** illustrates visual outputs. **1701** illustrates a visual display outputting data in real-time. **1701a** illustrates a screen that displays sequencing assay parameters processed in real-time. Example output parameters include: a) median depth of sequencing; b) standard-deviation of depth of sequencing; c) total number of sequencing-reads received; d) percentage of pair aligned reads; e) percentage of single aligned reads; f) percentage of pair unaligned reads; g) percentage of zero coverage; h) percentage low coverage; i) mean insert size; and j) standard deviation of the insert size.

Display module **1701b** illustrates displays parameters associated with barcode and sequencing read assembly in real-time. Examples of display information include: a) a total number of unique barcodes; b) percentage of barcodes with errors; c) final number of unique barcodes; d) mean reads per barcode; e) percentage of reads that are realigned; f) mean inferred contiguous sequence length; g) percentage of inferred contiguous sequences smaller than a threshold size; h) number of inferred contiguous sequences; i) mean or maximum contiguous sequence length per barcode; and j) standard deviation of contiguous sequence per barcode. Display module **1701c** displays parameters associated with polymorphisms in real-time. Example parameters include: a) number of single nucleotide polymorphisms identified; b) number of INDELS identified; c) rate of identifying homozygous polymorphisms; d) number of translocations identified; e) number of translocations associated with a haplotype; % single nucleotide polymorphisms associated with a haplotype; and g) rate of INDELS associated with a haplotype.

The output modules can display graphical representations of the parameters above. **1701d** illustrates a graphical representation of a coverage depth of sequencing. **1701e** illustrates a graphical representation of an inferred assembly of a set of sequencing reads. **1701f** illustrates a graphical representation of the length of a phase block.

Data Storage.

Output data from a sequencing experiment, and output results from data analysis, can be stored electronically in data archives. Any information described herein can be stored in a data archive, and specific information can be stored in files configured to contain or reference any barcodes associated with the information. A record can contain, for example, information associated with a variant that was identified in data analysis. Such information can include the identifying features of the variant, such as the chromosome bearing the variant, the position on the chromosome, and the identities of the nucleobases varied from a reference, for example, replacing adenosine with cytidine.

The record can contain the identities or sequences of any sequencing read associated with the variant. The record can further contain any barcode associated with the variant or associated sequencing reads. The inclusion of the barcodes in the records provides access to any information derivable from the barcodes, such as the error probability associated with the variant, and haplotype phasing.

The incorporation or association of barcodes with the records describing the variants allows for significant improvements in the efficiency of data analysis and manipulation. A user has access to the relevant barcodes without the need to search the raw data to find the barcodes associated with the variant. If further data analysis is done at a later time, the analysis can begin directly from the record rather than having to redo the initial data analysis. The avoidance of reanalyzing barcode/variant correlations established previously saves time and cost, and facilitates the performance of multiple successive analyses from the same data batch. Also, a user can focus subsequent data analysis on precisely the barcodes, or sequencing reads associated therewith, relevant to the variant of interest without the need to process all other barcodes associated with the original sample material. The data storage system promotes multiple bioinformatic applications, such as variant phasing, single-cell analysis, and metagenomics by using the barcodes to identify the place of origin of a variant.

FIG. **21** illustrates a non-limiting example of the record system. **2101** illustrates obtaining the raw sequencing data in the form of a Fastq file. The Fastq file can contain many

barcodes associated with a sequencing experiment. Alignment of the sequencing reads provides aligned data, which can be stored in a BAM file (2102). The barcodes can be associated with variants in the aligned data, and can identify the variant by location. 2102 shows that barcode 1 is associated with a variant at position 3,955,443 on chromosome 20, and barcode 2 is associated with a variant at position 41,405,930 on chromosome 3. In 2103, the variants have been called and recorded in a VCF file. The VCF file identifies the differences between the sequencing data and a reference. Position 3,955,443 on chromosome 20 of the sample has a cytidine residue, whereas the reference has an adenosine residue, and position 41,405,930 on chromosome 3 of the sample has an adenosine residue whereas the reference has a guanosine residue. Once the variants are identified, the record can be elaborated (through use of the bam file (2102)) with the identities of all barcodes that support the variant call, including barcodes supporting the called variant and barcodes supporting the reference allele (2104).

Generating and Analyzing DNA/RNA Sequencing Reads.

Sequencing experiments can be done individually or in parallel to provide tens, hundreds, or thousands of sequences simultaneously. In addition to identifying standard nucleotides and amino acids, methods herein can identify natural and non-natural modifications within a sample, for example, methylation of the cytosine base to produce 5-methylcytosine. In some embodiments, a sample comprises modifications within traditional base pairs, such as hypoxanthine, or xanthine, which can be purine derivatives. In some embodiments, a sample can include natural and non-natural RNA derivatives, such as Inosine (I), and modifications such as 2'-O-methylribose modifications.

A computer program products and methods herein can analyze data generated by any sequencing instrument. Non-limiting examples of sequencers include: a) DNA sequencers produced by Illumina™, for example, HiSeq™, HiScanSQ™, Genome Analyzer GAII™, and MiSeq™ models; b) DNA sequencers produced by Life Technologies™, for example, DNA sequencers under the AB Applied Biosystems™ and/or Ion Torrent™ brands; c) DNA sequencers manufactured by Beckman Coulter™; d) DNA sequencers manufactured by 454 Life Sciences™; and e) DNA sequencers manufactured by Pacific Biosciences™.

Methods of the invention can analyze data obtained in a variety of sequencing experiments. For example, sequencing-by-synthesis technology uses a unique fluorescent-label for each of adenine, cytidine, guanine, and thymidine. A nucleotide chain is synthesized based on a sample sequence, and during each sequencing cycle, a single labeled deoxynucleoside triphosphate (dNTP) is added to the nucleic acid chain. The nucleotide label terminates polymerization. After each dNTP incorporation, the fluorescent dye is irradiated to identify the newly-added residue and then the label is enzymatically cleaved to allow incorporation of the next nucleotide.

Semiconductor sequencing technology is based on the premise that when a nucleotide is incorporated into a strand of DNA by a polymerase, a hydrogen ion is released as a byproduct. A sequence is synthesized based on a template sample sequence. Each of adenine, cytidine, guanine, and thymidine is added sequentially. If one of the nucleotides is incorporated into the growing chain, the charge from the released hydrogen ion changes the pH of the reaction solution. A solid-state pH meter detects the pH change, thereby identifying the chain elongation. If a nucleotide is not incorporated, no voltage change will be recorded by the

solid-state pH meter and no information will be incorporated into a sequencing read. A pattern of released hydrogen ions can provide the information needed to construct a sequencing read.

5 Samples.

A sequencing read can be generated from various samples. A sample can be derived from a variety of biological sources, such as blood, saliva, tissue, or a biopsy. A sequence of a sample can comprise information associated with a variety of biomolecules. Non-limiting examples of biomolecules include amino acids, peptides, peptide-mimetics, proteins, recombinant proteins, antibodies (monoclonal or polyclonal), antibody fragments, antigens, epitopes, carbohydrates, lipids, fatty acids, enzymes, natural products, nucleic acids (including DNA, mRNA, microRNA, tRNA, rRNA, long non-coding RNAs, snoRNA, nucleosides, nucleotides, structure analogues or combinations thereof), nutrients, receptors, and vitamins.

In some embodiments, the amount of sample requires to provide sufficient data for analysis, or analysis with a the confidence rates or error rates described herein, is no more than about 1 ng, no more than about 5 ngs, no more than about 10 ngs, no more than about 15 ngs, no more than about 20 ngs, no more than about 25 ngs, no more than about 30 ngs, no more than about 35 ngs, no more than about 40 ngs, no more than about 45 ngs, no more than about 50 ngs, no more than about 55 ngs, no more than about 60 ngs, no more than about 65 ngs, no more than about 70 ngs, no more than about 75 ngs, no more than about 80 ngs, no more than about 85 ngs, no more than about 90 ngs, no more than about 95 ngs, no more than about 100 ngs, no more than about 200 ngs, no more than about 300 ngs, no more than about 400 ngs, no more than about 500 ngs, no more than about 600 ngs, no more than about 700 ngs, no more than about 800 ngs, no more than about 900 ngs, or no more than about 1 mg. In some embodiments, the amount of sample requires to provide sufficient data for analysis, or analysis with a the confidence rates or error rates described herein, is no more than about 1 pg, no more than about 5 pgs, no more than about 10 pgs, no more than about 15 pgs, no more than about 20 pgs, no more than about 25 pgs, no more than about 30 pgs, no more than about 35 pgs, no more than about 40 pgs, no more than about 45 pgs, no more than about 50 pgs, no more than about 55 pgs, no more than about 60 pgs, no more than about 65 pgs, no more than about 70 pgs, no more than about 75 pgs, no more than about 80 pgs, no more than about 85 pgs, no more than about 90 pgs, no more than about 95 pgs, no more than about 100 pgs, no more than about 200 pgs, no more than about 300 pgs, no more than about 400 pgs, no more than about 500 pgs, no more than about 600 pgs, no more than about 700 pgs, no more than about 800 pgs, no more than about 900 pgs, or no more than about 1 ng.

DNA Sequencing Sample Preparation.

55 A non-limiting example of a comprehensive DNA sequencing protocol that generates sequencing-reads is as follows.

Sample preparation can include DNA shearing. DNA shearing can comprise: (1) Transferring 1-3 µg of DNA to a 1.5 mL tube; (2) Bringing the DNA up to a volume of up to 130 µL with nuclease-free water; and (3) Transferring DNA to a microtube in preparation for sonication at 4° C. for 1-10 minutes

The sheared sample can be immobilized on a surface. Solid Phase Reversible Immobilization (SPRI™) beads can be used to immobilize a DNA or an RNA sample to a surface. The beads can be paramagnetic beads coated with

nucleic acid binding moieties, such as carboxyl groups. To immobilize DNA to a set of beads, a user can: (1) Mix by vortex a quantity of beads to ensure a homogeneous solution; (2) Transfer 180 μL of bead solution to a 1.5 mL tube. (3) Add 100 μL of end-repaired DNA; (4) Mix by vortex for 5 minutes; (5) Place the tube on a magnet for 3-5 minutes, until the solution is clear; (6) Remove the cleared supernatant, while tube is on the magnet; (7) Add approximately 500 mL of 70% ethanol, while the tube is on the magnet; (8) Allow the beads to settle for approximately 1 minute; (9) Remove the ethanol and wash once with 500 μL of 70% ethanol; (10) Remove the ethanol; (11) Dry the sample on a heating block at 37° C. for 5 minutes to remove residual ethanol; (12) Add 10 μL of nuclease-free water to the beads; (13) Mix by vortex and incubate at room temperature for 2 minutes; (14) Place the tube on magnet for 2-3 minutes until the solution is clear; and (15) Transfer 15 μL of the solution to a fresh PCR tube, while being careful not to disturb the beads.

Sample preparation can include steps to add supplementary adenosine bases to immobilized/fragmented DNA. To add adenosine bases to immobilized/fragmented DNA, a user can: (1) Prepare a reaction (10 μL eluted DNA; 2 μL 10 \times Blue Buffer; 5 μL 1 mM dATP; and 3 μL Klenow exo-50,000 U/mL) in a 1.5 mL tube; (2) Incubate in the tube in a heat block at 37° C. for 30 minutes; and (3) Heat-inactivate the enzyme by incubating at 75° C. for 20 minutes.

Sample preparation can comprise ligating adapters to DNA fragments. An adapter can comprise a barcode. An adapter can be a structure used to attach a barcode to a target polynucleotide. A sample of DNA or RNA (total or fractionated, such as poly(A) enriched RNA) can be converted to a library of fragments with adaptors attached to one or both ends.

A non-limiting example of a protocol that can ligate an adapter to DNA fragments includes: (1) Prepare a reaction (20 μL DNA; 25 μL 2 \times rapid ligase buffer; 100 μM adapter mix; and 1 μL T4 DNA ligase) in a 1.5 mL tube; (2) Incubate on a thermal cycler at 20° C. for 30 minutes; and (3) Purify with AMPure™ XP beads.

The DNA-adapted ligated fragments can be purified prior to sequencing. A non-limiting example of a method of purification includes AMPure™ DNA Purification methods. (1) SPRI™ beads can be allowed to equilibrate at room temperature. (2) Beads are mixed by vortex to ensure a homogeneous solution. (3) 60 μL of bead solution is transferred to a 1.5 mL tube. (4) 50 μL of the adapter-ligated library is transferred to the tube. (5) Mix by vortex for 5 minutes. (6) The tube is placed on a magnet for 3-5 minutes, until the solution is clear. (7) The supernatant is removed, while the tube is on the magnet. (8) 500 μL of 70% ethanol is added to the sample while the tube is on the magnet. (9) Beads are allowed to settle for 1 minute. (10) Ethanol is removed and the beads are washed again with 500 μL of 70% ethanol. (11) Ethanol is removed. (12) The sample is dried on a 37° C. heat block for 5 minutes to remove residual ethanol. (13) Add 30 μL of nuclease-free water to the beads. (14) The sample is mixed by vortex and incubated at room temperature for about 2 minutes. (15) The tube is placed on a magnet for about 2-3 minutes until the solution is clear. (16) 30 μL of solution is transferred to fresh PCR tubes. To confirm the addition of adapters to DNA samples, 1 μL of sample can be evaluated on, for example, an Agilent 2100 Bioanalyzer™.

A non-limiting example of a protocol for sample library amplification includes: (1) Prepare a reaction (15 μL purified

DNA template; 1.5 μL 25 μM forward primer; 1.5 μL 25 μM reverse primer; 25 μL 2 \times Mastermix; and 7 μL water) for each sample to be amplified. (2) Run a thermal cycler program (A: 98° C. for 2 minutes; B: 11 cycles of: i) 98° C. for 20 seconds; ii) 64° C. for 30 seconds; and iii) 72° C. for 5 minutes; C: 72° C. for 5 minutes; and D: hold at 4° C.). (3) Purify sample.

RNA Sequencing Sample Preparation.

A method and a computer program product of the invention can analyze RNA sequencing data. A non-limiting example of a protocol generating RNA sequencing reads is the following: Whole transcriptome RNA can be isolated and a library can be prepared for deep sequencing analysis.

Library Construction: (1) mRNA is purified from Total RNA using magnetic beads: 5-100 ng of total RNA is diluted with nuclease-free water and heated to disrupt the secondary structures and then added to pre-prepared Magnetic Oligo (dT) Beads. After washing with washing buffer, 10 mM Tris-HCl is added to the beads and mRNA is eluted from the

beads. (2) Fragmentation of the mRNA: The mRNA is fragmented into small pieces using divalent cations under elevated temperature. (3) Synthesis of the first and second strands of cDNA: The cleaved RNA fragments are copied into a first strand of cDNA using reverse transcriptase and

random primers. Then the primary strand of mRNA is removed by RNaseH and a replacement strand is synthesized to generate double-stranded cDNA. (4) Overhang end repair into blunt ends: The overhangs resulting from fragmentation are converted into blunt ends using T4 DNA

polymerase and *E. coli* DNA polymerase I Klenow fragment. The 3' to 5' exonuclease activity of these enzymes removes 3' overhangs, and the polymerase activity fills in the 5' overhangs. (5) Addition of 'A' Bases to the 3' End of the DNA Fragments: An 'A' base is added to the 3' end of the

blunt phosphorylated DNA fragments, using the polymerase activity of Klenow fragment (3' to 5' exo minus). This step prepares the DNA fragments for ligation to the adapters, which have a single 'T' base overhang at the 3' end. (6) Ligation of Adapters to DNA Fragments: The adapters are

ligated to the ends of the DNA fragments, preparing DNA fragments to be hybridized to a flow cell. (7) Purification of Ligation Products: The products of the ligation reaction are purified on a 2% TAE-agarose gel to remove all unligated adapters and any adapters that have ligated to one another, and selected in a size-range of templates (for example

200 \pm 25 bp) for downstream enrichment. (8) Enrichment of the Adapter-cDNA Fragments by PCR: The cDNA fragments with adapters on both ends are amplified for 15 cycles of PCR, with two primers complementary to the ends of the

adapters. (9) Validate the Library: Check the size, purity, and concentration of the library constructs using standard methods.

Template hybridization, cluster amplification, linearization, blocking, and primer hybridization: (1) An example of a sequencing apparatus is the cBot™ fluidics device that hybridizes samples onto a flow cell and amplifies for later sequencing on the HiSeq2000/1000™. The instrument uses solid support amplification to create an ultra-high density sequencing flow cell with millions of clusters, each containing 1,000 copies of template. Prior to sequencing, an initial in vitro clonal amplification step is used. The bridge PCR for cluster generation is achieved via the following steps: (2) Denaturation of the library construct and hybridization of the template DNA: Original dsDNA construct is denatured

and converted to ssDNA that already has adapters ligated thereto, and is ready to hybridize via the homology of the adapters to a complementary adaptor primer on a grafted

flowcell. (3) Amplification of template DNA: the template DNA is isothermally amplified to form a surface-bound colony (the clonal DNA cluster). (4) Linearization: dsDNA clusters are linearized as the first step of converting dsDNA to ssDNA that is suitable for sequencing. (5) Blocking: the free 3' OH ends of the linearized dsDNA clusters are blocked. This step prevents nonspecific sites from being sequenced. (6) Denaturation and hybridization of sequencing primers: in this step the denatured dsDNA is then hybridized to a sequencing primer, or multiple sequencing primers, onto the linearized and blocked clusters. After this step, the flow cell is ready for sequencing.

Reference Sequences, Genomes, and Databases.

A computer program product and a method of the invention can analyze sequencing reads drawn from a database. A database can provide DNA, RNA, or protein sequencing data. A database comprising sequencing information can be a public database or a private database. A database can be specialized in a particular type of digital sequencing read data, or can be a central database comprising a plurality of distinct data formats. Non-limiting examples of databases include the NCBI completely sequenced genomes database, the *Saccharomyces* Genome database, FlyBase, WormBase, *Arabidopsis* Information Resource, Zebrafish Information Network, Swiss-Prot, TrEMBL, Protein Information Resource, Uniprot, 1000 Genomes Project database, and a database of the International HapMap Project.

A reference genome can be assembled as a representative reference sequence of a gene, a set of genes, or of the genome of a creature. A creature can be any creature in the six kingdoms of a biological taxonomical rank: Animalia, Plantae, Fungi, Protista, Archaea, and Bacteria. A creature can be, for example, a Human (*H. sapiens*), a Mouse (*M. musculus*), a Rat (*R. norvegicus*), a Fly (*D. melanogaster*), a Fly (*D. pseudoobscura*), a Fly (*D. pmelanogaster*-multiple strains), a Honey bee (*A. mellifera*), a Sea urchin (*S. purpuratus*), Bovine (*B. taurus*), a Rhesus monkey (*M. mulatta*), an Orangutan (*P. pygmaeus*), a Marmoset (*C. jacchus*), a Pea aphid (*A. pisum*), a Wasp (*Nasonia* spp.), a Beetle (*T. castaneum*), a Wallaby (*M. eugenii*), an Acorn worm (*S. kowalevskii*), a Hyrax (*P. capensis*), a Megabat (*P. vampyrus*), an Armadillo (*D. novemcinctus*), a Baboon (*P. anubis*), a Bumble bee (*B. terrestris*), a California mouse (*P. californicus*), a Centipede (*S. maritima*), a Cotton bollworm (*H. armigera*), a Deer mouse (*P. maniculatus*), a Gibbon, a Dolphin, Bottlenose (*T. truncatus*), a Dwarf honey bee (*A. florea*), maize (*Z. mays*), or any other creature.

A digital sequence of a reference sequence can be contained in a database of reference sequences. A database of reference sequences can comprise a plurality of genomes and/or proteomes, sequenced with a plurality of methods. A reference genome can be a haploid, a diploid, or a polyploid genome. In some embodiments, the reference genome is a human genome. In some embodiments, the reference genome is an annotated human genome. In some embodiments, a system, a method, an algorithm, and/or a computer program product of the invention can identify a mutation and/or a variant in a genomic sample based on a comparison of an experimentally-generated sequencing read with a reference genome.

A reference sequence can be a digital nucleic acid or amino acid sequence. A reference genome can be a reference sequence. A reference genome can comprise digital annotations of a known allele, even if the known allele is not present in either the physical or the digital version of the nucleic acid sequence. For example, humans have the A, B, AB, or O blood type as designated in the ABO blood system. A reference sequence containing only an O allele can be annotated with alleles corresponding to the A, B, and AB gene sequences.

Polymorphisms.

A large number of variants have been identified in sequenced genomes, including the human genome. Variants can be of functional significance, for example, leading to a missense mutation or a non-sense mutation in a protein coding gene. Variants can be of no functional significance, such as silent mutations. Single nucleotide polymorphisms (SNPs) can be of functional significance.

In some embodiments, the invention provided herein can identify polymorphisms present in a genome. In some embodiments, the invention can associate an identified polymorphism with a condition. In some embodiments polymorphisms identified in a genome as compared to a reference genome represents from about 0.1% to about 0.05%, from about 0.1% to about 0.01%, from about 0.05% to about 0.01%, from about 0.05% to about 0.005%, from about 0.01% to about 0.005%, or from about 0.005% to about 0.0001% of a sequenced genome. In some embodiments, the invention identifies a mutation in a sample based on a comparison with a reference sequence.

TABLE 3 illustrates the physical organization and the number of polymorphisms reported within a human genome by chromosome.

TABLE 3

Chromosome	Length (mm)	Base Pairs	No. of Reported Polymorphisms	Protein coding genes	Pseudo-genes	Micro RNA	Ribosomal RNA	Small nuclear RNA
1	85	249,250,621	4,401,091	2,012	1,130	134	66	221
2	83	243,199,373	4,607,702	1,203	948	115	40	161
3	67	198,022,430	3,894,345	1,040	719	99	29	138
4	65	191,154,276	3,673,892	718	698	92	24	120
5	62	180,915,260	3,436,667	849	676	83	25	106
6	58	171,115,067	3,360,890	1,002	731	81	26	111
7	54	159,138,663	3,045,992	866	803	90	24	90
8	50	146,364,022	2,890,692	659	568	80	28	86
9	48	141,213,431	2,581,827	785	714	69	19	66
10	46	135,534,747	2,609,802	745	500	64	32	87
11	46	135,006,516	2,607,254	1,258	775	63	24	74
12	45	133,851,895	2,482,194	1,003	582	72	27	106
13	39	115,169,878	1,814,242	318	323	42	16	45
14	36	107,349,540	1,712,799	601	472	92	10	65
15	35	102,531,392	1,577,346	562	473	78	13	63

TABLE 3-continued

Chromosome	Length (mm)	Base Pairs	No. of Reported Polymorphisms	Protein coding genes	Pseudo-genes	Micro RNA	Ribosomal RNA	Small nuclear RNA
16	31	90,354,753	1,747,136	805	429	52	32	53
17	28	81,195,210	1,491,841	1,158	300	61	15	80
18	27	78,077,248	1,448,602	268	59	32	13	51
19	20	59,128,983	1,171,356	1,399	181	110	13	29
20	21	63,025,520	1,206,753	533	213	57	15	46
21	16	48,129,895	787,784	225	150	16	5	21
22	17	51,304,566	745,778	431	308	31	5	23
X	53	155,270,560	2,174,952	815	780	128	22	85
Y	20	59,373,566	286,812	45	327	15	7	17
Mt. DNA	0.0054	16,569	929	13	0	0	2	0

Therapies.

Integrating inherited genetic risk information into the clinical decision-making process early in life can have an important effect in ameliorating or even preventing disease symptoms or conditions. The computer program products and methods of the invention address pressing challenges in personalized medicine. By providing a streamlined and rapid method for effective interpretation and characterization of data generated in sequencing experiments, the invention can solve challenges in conveying meaningful interpretations of a genomic or proteomic sequence to physicians. The invention can facilitate characterization of various genetic conditions based on sequencing data.

Subjects can be afflicted by one or a plurality of conditions, or subjects can be healthy. Subjects can be of any age, including, for example, elderly adults, adults, adolescents, pre-adolescents, children, toddlers, and infants. Non limiting examples of subjects include humans, other primates, and mammals such as dogs, cats, horses, pigs, rabbits, rats, and mice.

In some embodiments, the invention can identify a condition based on an analysis of sequencing data associated with a sample.

A computer-program product and a method of the invention can identify genetic signatures associated with various conditions. Non-limiting examples of conditions include cystic fibrosis, Duchenne muscular dystrophy, Haemochromatosis, Tay-Sachs disease, Prader-Willi syndrome, Angelman syndrome, neurofibromatosis, phenylketonuria, Canavan disease, Coeliac disease, Acid beta-glucosidase deficiency, Gaucher, Charcot-Marie-Tooth disease, color blindness, Cri du chat, polycystic kidney disease, acrocephaly, familial adenomatous polyposis, adrenal gland disorders, amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, anemia, ataxia, ataxia telangiectasia, autism, bone marrow diseases, Bonnevie-Ullrich syndrome, brain diseases, von Rippel-Lindau disease, congenital heart disease, Crohn's disease, dementia, myotonic dystrophy, Fabry disease, fragile X syndrome, galactosemia, genetic emphysema, retinoblastoma, Pendred syndrome, Usher syndrome, Wilson disease, neuropathies, Huntington's disease, immune system disorders, gout, X-linked spinal-bulbar muscle atrophy, learning disabilities, Li-Fraumeni syndrome, lipase D deficiency, Lou Gehrig disease, Marfan syndrome, metabolic disorders, Niemann-Pick, Noonan syndrome, Osteopsathyrosis, Peutz-Jeghers syndrome, Pfeiffer syndrome, porphyria, progeria, Rett syndrome, tuberous sclerosis, speech and communication disorders, spinal muscular atrophy, Treacher Collins syndrome, trisomies, and monosomies.

A computer-program product, a method, an algorithm, or a system herein can identify variants present in a various cancers. Non-limiting examples of cancers include: acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytomas, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancers, brain tumors, such as cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas, Burkitt lymphoma, carcinoma of unknown primary origin, central nervous system lymphoma, cerebellar astrocytoma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, germ cell tumors, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gliomas, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, Hypopharyngeal cancer, intraocular melanoma, islet cell carcinoma, Kaposi sarcoma, kidney cancer, laryngeal cancer, lip and oral cavity cancer, liposarcoma, liver cancer, lung cancers, such as non-small cell and small cell lung cancer, lymphomas, leukemias, macroglobulinemia, malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma, melanomas, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, myelodysplastic syndromes, myeloid leukemia, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, pancreatic cancer, pancreatic cancer islet cell, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pituitary adenoma, pleuropulmonary blastoma, plasma cell neoplasia, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma, renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcomas, skin cancers, skin carcinoma merkel cell, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, stomach cancer, T-cell lymphoma, throat cancer, thymoma, thymic carcinoma, thyroid cancer, trophoblastic tumor (gestational), cancers of unknown primary site, urethral

cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenström macroglobulinemia, and Wilms tumor.

The invention can further comprise recommending or providing a therapy to a subject based on an analysis of sequencing data. A therapy can be a medicinal intervention, such as a chemotherapy, or a medical procedure, such as a surgery. For example, an analysis of sequencing data can identify a polymorphism known to contribute to cystic fibrosis in a sample. Non-limiting examples of therapeutic interventions that can be provided to a subject afflicted by cystic fibrosis include antibiotics, anti-inflammatories, enzyme replacement, mucolytics, airway clearance, postural drainage, wearing an inflatable therapy vest, using a flutter device, diet regimens, exercise regimens, and organ transplants.

The therapy provided to the subject can be directed towards a genetic component of an identified condition. For example, mutations in the Abelson tyrosine kinase (ABL) gene can lead to Chronic Myeloid Leukemia (CML), and single nucleotide polymorphisms can render subjects resistant to Gleevec. An analysis of sequencing data associated with a sample can identify a polymorphisms associated with CML, and a physician can use an output to determine that the subject is potentially non-responsive to Gleevec.

Data Processing and Error Rates.

Non-limiting examples of real-time analysis time-frames for a comparison of sequencing reads include: less than 1 second, less than 2 seconds, less than 3 seconds, less than 4 seconds, less than 5 seconds, less than 10 seconds, less than 15 seconds, less than 30 seconds, less than 45 seconds, less than 60 seconds; less than 2 minutes; less than 3 minutes; less than 4 minutes; less than 5 minutes; less than 6 minutes; less than 7 minutes; less than 8 minutes; less than 9 minutes; less than 10 minutes; less than 11 minutes; less than 12 minutes; less than 13 minutes; less than 14 minutes; less than 15 minutes; less than 16 minutes; less than 17 minutes; less than 18 minutes; less than 19 minutes; less than 20 minutes; less than 21 minutes; less than 22 minutes; less than 23 minutes; less than 24 minutes; less than 25 minutes; less than 26 minutes; less than 27 minutes; less than 28 minutes; less than 29 minutes; and less than 30 minutes.

Non-limiting examples of real-time analysis time-frames of complete sequencing experiments include less than 15 minutes; from about 10 minutes to about 30 minutes; from about 10 minutes to about 1 hour; from about 10 minutes to about 2 hours; from about 10 minutes to about 3 hours; from about 10 minutes to about 4 hours; from about 10 minutes to about 5 hours; from about 10 minutes to about 6 hours; from about 10 minutes to about 7 hours; from about 10 minutes to about 8 hours; from about 10 minutes to about 9 hours; from about 10 minutes to about 10 hours; from about 1 hour to about 2 hours; from about 1 hour to about 3 hours; from about 1 hour to about 4 hours; from about 1 hour to about 5 hours; from about 1 hour to about 6 hours; from about 1 hour to about 7 hours; from about 1 hour to about 8 hours; from about 1 hour to about 9 hours; from about 1 hour to about 10 hours; from about 2 hours to about 3 hours; from about 2 hours to about 4 hours; from about 2 hours to about 5 hours; from about 2 hours to about 6 hours; from about 2 hours to about 7 hours; from about 2 hours to about 8 hours; from about 2 hours to about 9 hours; from about 2 hours to about 10 hours; from about 3 hours to about 4 hours; from about 3 hours to about 5 hours; from about 3 hours to about 6 hours; from about 3 hours to about 7 hours; from about 3 hours to about 8 hours; from about 3 hours to about 9 hours; from about 3 hours to about 10 hours; from about 4 hours to about 5 hours; from about 4 hours to about 6

hours; from about 4 hours to about 7 hours; from about 4 hours to about 8 hours; from about 4 hours to about 9 hours; from about 4 hours to about 10 hours; from about 5 hours to about 6 hours; from about 5 hours to about 7 hours; from about 5 hours to about 8 hours; from about 5 hours to about 9 hours; from about 5 hours to about 10 hours; from about 6 hours to about 7 hours; from about 6 hours to about 8 hours; from about 6 hours to about 9 hours; from about 6 hours to about 10 hours; from about 7 hours to about 8 hours; from about 7 hours to about 9 hours; from about 7 hours to about 10 hours; from about 8 hours to about 9 hours; from about 8 hours to about 10 hours; from about 9 hours to about 10 hours; and about 10 hours.

A method of identifying sequencing errors can comprise comparing a first sequencing read, with a number of additional sequencing reads. The additional sequencing reads can comprise a common barcode or share a level of homology with the first sequencing read or one another. In some embodiments, a confidence level of determining that a variant in a sequencing assay is an error increases as the number of additional sequencing reads compared to the first sequencing read increases.

In some embodiments, a computer program product and an algorithm of the invention can detect, process, distinguish, and group at least 100 barcodes per sample; at least 500 barcodes per sample; at least 1,000 barcodes per sample; at least 2,000 barcodes per sample; at least 3,000 barcodes per sample; at least 4,000 barcodes per sample; at least 5,000 barcodes per sample; at least 6,000 barcodes per sample; at least 7,000 barcodes per sample; at least 8,000 barcodes per sample; at least 9,000 barcodes per sample; at least 10,000 barcodes per sample; at least 11,000 barcodes per sample; at least 12,000 barcodes per sample; at least 13,000 barcodes per sample; at least 14,000 barcodes per sample; at least 15,000 barcodes per sample; at least 16,000 barcodes per sample; at least 17,000 barcodes per sample; at least 18,000 barcodes per sample; at least 19,000 barcodes per sample; at least 20,000 barcodes per sample; at least 21,000 barcodes per sample; at least 22,000 barcodes per sample; at least 23,000 barcodes per sample; at least 24,000 barcodes per sample; at least 25,000 barcodes per sample; at least 26,000 barcodes per sample; at least 27,000 barcodes per sample; at least 28,000 barcodes per sample; at least 29,000 barcodes per sample; at least 30,000 barcodes per sample; at least 31,000 barcodes per sample; at least 32,000 barcodes per sample; at least 33,000 barcodes per sample; at least 34,000 barcodes per sample; at least 35,000 barcodes per sample; at least 36,000 barcodes per sample; at least 37,000 barcodes per sample; at least 38,000 barcodes per sample; at least 39,000 barcodes per sample; at least 40,000 barcodes per sample; at least 41,000 barcodes per sample; at least 42,000 barcodes per sample; at least 43,000 barcodes per sample; at least 44,000 barcodes per sample; at least 45,000 barcodes per sample; at least 46,000 barcodes per sample; at least 47,000 barcodes per sample; at least 48,000 barcodes per sample; at least 49,000 barcodes per sample; at least 50,000 barcodes per sample; at least 51,000 barcodes per sample; at least 52,000 barcodes per sample; at least 53,000 barcodes per sample; at least 54,000 barcodes per sample; at least 55,000 barcodes per sample; at least 56,000 barcodes per sample; at least 57,000 barcodes per sample; at least 58,000 barcodes per sample; at least 59,000 barcodes per sample; at least 60,000 barcodes per sample; at least 61,000 barcodes per sample; at least 62,000 barcodes per sample; at least 63,000 barcodes per sample; at least 64,000 barcodes per sample; at least 65,000 barcodes per sample; at least 66,000 barcodes per sample; at least 67,000 barcodes per sample; at

ing read, about 39 nucleotides per sequencing read, or about 40 nucleotides per sequencing read.

An identified error rate within a sample can be about 0.0000000001%, about 0.0000000002%, about 0.0000000003%, about 0.0000000004%, about 0.0000000005%, about 0.0000000006%, about 0.0000000007%, about 0.0000000008%, about 0.0000000009%, about 0.000000001%, about 0.000000002%, about 0.000000003%, about 0.000000004%, about 0.000000005%, about 0.000000006%, about 0.000000007%, about 0.000000008%, about 0.000000009%, about 0.00000001%, about 0.00000002%, about 0.00000003%, about 0.00000004%, about 0.00000005%, about 0.00000006%, about 0.00000007%, about 0.00000008%, about 0.00000009%, about 0.0000001%, about 0.0000002%, about 0.0000003%, about 0.0000004%, about 0.0000005%, about 0.0000006%, about 0.0000007%, about 0.0000008%, about 0.0000009%, about 0.000001%, about 0.000002%, about 0.000003%, about 0.000004%, about 0.000005%, about 0.000006%, about 0.000007%, about 0.000008%, about 0.000009%, about 0.00001%, about 0.00002%, about 0.00003%, about 0.00004%, about 0.00005%, about 0.00006%, about 0.00007%, about 0.00008%, about 0.00009%, about 0.0001%, about 0.0002%, about 0.0003%, about 0.0004%, about 0.0005%, about 0.0006%, about 0.0007%, about 0.0008%, about 0.0009%, about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.1%.

Homology.

A sequencing read can be aligned to a reference sequence with a degree of homology. A first sequencing read can be aligned to a second sequencing read with a degree of homology. A portion (“window”) of a sequencing read can share a percent homology to a reference sequence, or a second sequencing read. The entirety of a sequencing read can share a percent homology to a reference sequence, or a second sequencing read. Modules of the invention can determine a statistical significance of an alignment based on a percent of shared homology between two sequences. A sequencing read can share structural and/or base-pair (“pairwise”) homology to a reference sequence or a second sequencing read. Homology can be measured over an entire sequence or a fragment of a sequence. Homology can be measured by a computer-executable algorithm, for example, the Basic Local Alignment Search Tool (BLAST).

A sequencing read and a reference sequence can share about 1% pairwise homology, about 2% pairwise homology, about 3% pairwise homology, about 4% pairwise homology, about 5% pairwise homology, about 6% pairwise homology, about 7% pairwise homology, about 8% pairwise homology, about 9% pairwise homology, about 10% pairwise homology, about 11% pairwise homology, about 12% pairwise homology, about 13% pairwise homology, about 14% pairwise homology, about 15% pairwise homology, about 16% pairwise homology, about 17% pairwise homology, about 18% pairwise homology, about 19% pairwise homology, about 20% pairwise homology, about 21% pairwise homology, about 22% pairwise homology, about 23% pairwise

homology, about 24% pairwise homology, about 25% pairwise homology, about 26% pairwise homology, about 27% pairwise homology, about 28% pairwise homology, about 29% pairwise homology, about 30% pairwise homology, about 31% pairwise homology, about 32% pairwise homology, about 33% pairwise homology, about 34% pairwise homology, about 35% pairwise homology, about 36% pairwise homology, about 37% pairwise homology, about 38% pairwise homology, about 39% pairwise homology, about 40% pairwise homology, about 41% pairwise homology, about 42% pairwise homology, about 43% pairwise homology, about 44% pairwise homology, about 45% pairwise homology, about 46% pairwise homology, about 47% pairwise homology, about 48% pairwise homology, about 49% pairwise homology, about 50% pairwise homology, about 51% pairwise homology, about 52% pairwise homology, about 53% pairwise homology, about 54% pairwise homology, about 55% pairwise homology, about 56% pairwise homology, about 57% pairwise homology, about 58% pairwise homology, about 59% pairwise homology, about 60% pairwise homology, about 61% pairwise homology, about 62% pairwise homology, about 63% pairwise homology, about 64% pairwise homology, about 65% pairwise homology, about 66% pairwise homology, about 67% pairwise homology, about 68% pairwise homology, about 69% pairwise homology, about 70% pairwise homology, about 71% pairwise homology, about 72% pairwise homology, about 73% pairwise homology, about 74% pairwise homology, about 75% pairwise homology, about 76% pairwise homology, about 77% pairwise homology, about 78% pairwise homology, about 79% pairwise homology, about 80% pairwise homology, about 81% pairwise homology, about 82% pairwise homology, about 83% pairwise homology, about 84% pairwise homology, about 85% pairwise homology, about 86% pairwise homology, about 87% pairwise homology, about 88% pairwise homology, about 89% pairwise homology, about 90% pairwise homology, about 91% pairwise homology, about 92% pairwise homology, about 93% pairwise homology, about 94% pairwise homology, about 95% pairwise homology, about 96% pairwise homology, about 97% pairwise homology, about 98% pairwise homology, about 99% pairwise homology, or about 100% pairwise homology to a reference sequence or a second sequencing read.

An algorithm can allow for gaps in an alignment. An alignment gap can be of about 1 residue, about 2 residues, about 3 residues, about 4 residues, about 5 residues, about 6 residues, about 7 residues, about 8 residues, about 9 residues, about 10 residues, about 11 residues, about 12 residues, about 13 residues, about 14 residues, about 15 residues, about 16 residues, about 17 residues, about 18 residues, about 19 residues, about 20 residues, about 21 residues, about 22 residues, about 23 residues, about 24 residues, about 25 residues, about 26 residues, about 27 residues, about 28 residues, about 29 residues, about 30 residues, about 31 residues, about 32 residues, about 33 residues, about 34 residues, about 35 residues, about 36 residues, about 37 residues, about 38 residues, about 39 residues, about 40 residues, about 41 residues, about 42 residues, about 43 residues, about 44 residues, about 45 residues, about 46 residues, about 47 residues, about 48 residues, about 49 residues, or about 50 residues. In some embodiments, an alignment gap can be more than 50 residues.

Computer Architectures.

Sequencing data can be analyzed by a plurality of computers, with various computer architectures. Various com-

puter architectures are suitable for use with the invention. FIG. 18 is a block diagram illustrating a first example architecture of a computer system 1800 that can be used in connection with example embodiments of the present invention. As depicted in FIG. 18, the example computer system can include a processor 1802 for processing instructions. Non-limiting examples of processors include: Intel Core i7™ processor, Intel Core i5™ processor, Intel Core i3™ processor, Intel Xeon™ processor, AMD Opteron™ processor, Samsung 32-bit RISC ARM 1176JZ(F)-S v1.0™ processor, ARM Cortex-A8 Samsung S5PC100™ processor, ARM Cortex-A8 Apple A4™ processor, Marvell PXA 930™ processor, or a functionally-equivalent processor. Multiple threads of execution can be used for parallel processing. In some embodiments, multiple processors or processors with multiple cores can be used, whether in a single computer system, in a cluster, or distributed across systems over a network comprising a plurality of computers, cell phones, and/or personal data assistant devices.

As illustrated in FIG. 18, a high speed cache 1801 can be connected to, or incorporated in, the processor 1802 to provide a high speed memory for instructions or data that have been recently, or are frequently, used by processor 1802. The processor 1802 is connected to a north bridge 1806 by a processor bus 1805. The north bridge 1806 is connected to random access memory (RAM) 1803 by a memory bus 1804 and manages access to the RAM 1803 by the processor 1802. The north bridge 1806 is also connected to a south bridge 1808 by a chipset bus 1807. The south bridge 1808 is, in turn, connected to a peripheral bus 1809. The peripheral bus can be, for example, PCI, PCI-X, PCI Express, or other peripheral bus. The north bridge and south bridge are often referred to as a processor chipset and manage data transfer between the processor, RAM, and peripheral components on the peripheral bus 1809. In some architectures, the functionality of the north bridge can be incorporated into the processor instead of using a separate north bridge chip.

In some embodiments, system 1800 can include an accelerator card 1812 attached to the peripheral bus 1809. The accelerator can include field programmable gate arrays (FPGAs) or other hardware for accelerating certain processing.

Software and data are stored in external storage 1813 and can be loaded into RAM 1803 and/or cache 1801 for use by the processor. The system 1800 includes an operating system for managing system resources; non-limiting examples of operating systems include: Linux, Windows™, MACOS™, BlackBerry OS™, iOS™, and other functionally-equivalent operating systems, as well as application software running on top of the operating system.

In this example, system 1800 also includes network interface cards (NICs) 1810 and 1811 connected to the peripheral bus for providing network interfaces to external storage, such as Network Attached Storage (NAS) and other computer systems that can be used for distributed parallel processing.

FIG. 19 is a diagram showing a network 1900 with a plurality of computer systems 1902a, and 1902b, a plurality of cell phones and personal data assistants 1902c, and Network Attached Storage (NAS) 1901a, and 1901b. In some embodiments, systems 1902a, 1902b, and 1902c can manage data storage and optimize data access for data stored in Network Attached Storage (NAS) 1901a and 1902b. A mathematical model can be used for the data and be evaluated using distributed parallel processing across computer systems 1902a, and 1902b, and cell phone and personal data

assistant systems 1902c. Computer systems 1902a, and 1902b, and cell phone and personal data assistant systems 1902c can also provide parallel processing for adaptive data restructuring of the data stored in Network Attached Storage (NAS) 1901a and 1901b. FIG. 19 illustrates an example only, and a wide variety of other computer architectures and systems can be used in conjunction with the various embodiments of the present invention. For example, a blade server can be used to provide parallel processing. Processor blades can be connected through a back plane to provide parallel processing. Storage can also be connected to the back plane or as Network Attached Storage (NAS) through a separate network interface. In some embodiments, processors can maintain separate memory spaces and transmit data through network interfaces, back plane, or other connectors for parallel processing by other processors. In some embodiments, some or all of the processors can use a shared virtual address memory space.

FIG. 20 is a block diagram of a multiprocessor computer system using a shared virtual address memory space. The system includes a plurality of processors 2001a-f that can access a shared memory subsystem 2002. The system incorporates a plurality of programmable hardware memory algorithm processors (MAPs) 2003a-f in the memory subsystem 2002. Each MAP 2003a-f can comprise a memory 2004a-f and one or more field programmable gate arrays (FPGAs) 2005a-f. The MAP provides a configurable functional unit and particular algorithms or portions of algorithms can be provided to the FPGAs 2005a-f for processing in close coordination with a respective processor. In this example, each MAP is globally accessible by all of the processors for these purposes. In one configuration, each MAP can use Direct Memory Access (DMA) to access an associated memory 2004a-f, allowing it to execute tasks independently of, and asynchronously from, the respective microprocessor 2001a-f. In this configuration, a MAP can feed results directly to another MAP for pipelining and parallel execution of algorithms.

The above computer architectures and systems are examples only, and a wide variety of other computer, cell phone, and personal data assistant architectures and systems can be used in connection with example embodiments, including systems using any combination of general processors, co-processors, FPGAs and other programmable logic devices, system on chips (SOCs), application specific integrated circuits (ASICs), and other processing and logic elements. Any variety of data storage media can be used in connection with example embodiments, including random access memory, hard drives, flash memory, tape drives, disk arrays, Network Attached Storage (NAS) and other local or distributed data storage devices and systems.

In example embodiments, the computer system can be implemented using software modules executing on any of the above or other computer architectures and systems. In other embodiments, the functions of the system can be implemented partially or completely in firmware, programmable logic devices such as field programmable gate arrays (FPGAs) as referenced in FIG. 20, system on chips (SOCs), application specific integrated circuits (ASICs), or other processing and logic elements. For example, the Set Processor and Optimizer can be implemented with hardware acceleration through the use of a hardware accelerator card, such as accelerator card 1812 illustrated in FIG. 18.

EMBODIMENTS

In some embodiments, the invention provides a method of analyzing sequencing data associated with a sample, the

method comprising: a) receiving by a computer system a first sequencing read associated with a sequencing assay while the sequencing assay is in progress, wherein the computer system comprises a processor; and b) comparing by the processor the first sequencing read with another sequence to provide a comparison before the sequencing assay is complete. In some embodiments, the other sequence is a second sequencing read. Some embodiments further comprise determining whether the first sequencing read and the other sequence share a common subsequence. Some embodiments further comprise determining whether the first sequencing read and the other sequence correspond to a common biomolecule. Some embodiments further comprise aligning the first sequencing read and the other sequence. Some embodiments further comprise determining whether the first sequencing read and the other sequence share a common barcode. In some embodiments, the other sequence is a reference sequence. Some embodiments further comprise identifying a mutation in the sample based on the comparison. Some embodiments further comprise identifying a single nucleotide polymorphism in the sample based on the comparison. Some embodiments further comprise identifying a translocation in the sample based on the comparison. Some embodiments further comprise determining a candidate sequence, wherein the candidate sequence incorporates a portion of the first sequencing read. In some embodiments, determining the candidate sequence is performed at a rate, wherein the computer system receives a number of sequencing reads, wherein the rate increases as the number of sequencing reads increases. Some embodiments further comprise determining to which haplotype in a pair of haplotypes the first sequencing read corresponds. In some embodiments, the sample is associated with a plurality of distinct barcodes. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. In some embodiments, the sequencing data is peptide sequencing data. Some embodiments further comprise outputting a result of the comparing. Some embodiments further comprise providing a therapeutic intervention to a subject based on a result of analyzing the sequencing data. In some embodiments, the first sequencing read is a partial sequencing read. In some embodiments, the first sequencing read is a complete sequencing read.

In some embodiments, the invention provides a method of analyzing sequencing data associated with a sample, the method comprising: a) receiving by a computer system a set of sequencing reads, each sequencing read independently comprising a common barcode, wherein the computer system comprises a processor; and b) aligning by the processor each sequencing read of the set of sequencing reads against a reference sequence, wherein the sample is associated with a plurality of distinct barcodes. Some embodiments further comprise receiving an additional sequencing read that does not comprise the common barcode, and filtering out the additional sequencing read that does not comprise the common barcode. Some embodiments further comprise determining a candidate sequence based on the alignment of the sequencing reads against the reference sequence. Some embodiments further comprise identifying neighboring sequencing reads in the alignment, and determining whether the neighboring sequencing reads overlap at a non-barcode subsequence. Some embodiments further comprise determining whether the sequencing reads that comprise the common barcode correspond to a common haplotype. In some embodiments, the determining whether the sequencing

reads that comprise the common barcode correspond to the common haplotype comprises identifying a non-barcode subsequence common to a plurality of the sequencing reads that comprise the common barcode, and determining whether the sequencing reads of the plurality share a common single nucleotide polymorphism at the non-barcode common subsequence. Some embodiments further comprise determining a candidate sequence corresponding to one haplotype of a pair of haplotypes, wherein the candidate sequence is based on a plurality of sequencing reads, each of which comprise the common barcode, each of which share a common single nucleotide polymorphism at a common position. Some embodiments further comprise receiving a plurality of additional sequencing reads, wherein each additional sequencing read independently comprises a distinct barcode. Some embodiments further comprise aligning each additional sequencing read with the reference sequence. Some embodiments further comprise: c) receiving a plurality of additional sequencing reads, wherein each additional sequencing read independently comprises a distinct barcode; d) aligning each additional sequencing read with the reference sequence; e) selecting a window of the reference sequence; and f) determining a total of distinct barcodes associated with the additional sequencing reads that correspond to the window of the reference sequence. Some embodiments further comprise estimating a likelihood that the sample comprises a mutation. Some embodiments further comprise determining which haplotype in a pair of haplotypes possesses the mutation. In some embodiments, the mutation is a copy number variant. Some embodiments further comprise estimating the number of copies in the copy number variant. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. Some embodiments further comprise outputting a result of the aligning. Some embodiments further comprise providing a therapeutic intervention to a subject based on a result of analyzing the sequencing data.

In some embodiments, the invention provides a method of distinguishing a first haplotype and a second haplotype in a pair of haplotypes based on sequencing data, the method comprising: a) receiving by a computer system a first set of sequencing reads, wherein the computer system comprises a processor; b) determining by the processor that each sequencing read in the first set of sequencing reads possesses a common nucleic acid residue at a common position; and c) associating each sequencing read in the first set of sequencing reads with the first haplotype based on the possession of the common nucleic acid residue at the common position. In some embodiments, the common nucleic acid residue at the common position is a single nucleotide polymorphism. In some embodiments, the single nucleotide polymorphism is identified based on a comparison of the common nucleic acid residue at the common position with a corresponding position in a reference sequence. Some embodiments further comprise estimating a likelihood that one of the haplotypes possesses a mutation. In some embodiments, the mutation is a copy number variant. Some embodiments further comprise estimating the number of copies in the copy number variant. Some embodiments further comprise: d) receiving a second set of sequencing reads; e) determining that each sequencing read in the second set of sequencing reads possesses the common position and does not possess the common nucleic acid residue at the common position; and f) associating each sequencing read in the second set of sequencing reads with the second haplotype based on the non-possession of the

common nucleic acid residue at the common position. In some embodiments, each sequencing read in the first set of sequencing reads comprises a common barcode. In some embodiments, each sequencing read in the second set of sequencing reads comprises the common barcode. Some 5
embodiments further comprise: g) identifying a window of a reference sequence that corresponds to the sequencing reads in the first set of sequencing reads and the second set of sequencing reads; and h) comparing the total number of sequencing reads in the first set of sequencing reads that occur within the window and the total number of sequencing reads in the second set of sequencing reads that occur within the window. In some embodiments, the associating each sequencing read in the first set of sequencing reads with the first haplotype has an error rate of no greater than 1%. In 10
some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. Some embodiments further comprise outputting a result of the associating. Some 20
embodiments further comprise providing a therapeutic intervention to a subject based on a result of the method.

In some embodiments, the invention provides a method of analyzing sequencing data associated with a sample, the method comprising: a) receiving by a computer system a 25
first sequencing read, wherein the computer system comprises a processor; b) determining by the processor that the first sequencing read has a single polynucleotide polymorphism at a position; c) receiving a second sequencing read, wherein the second sequencing read comprises the position; 30
d) determining that the second sequencing read has the single polynucleotide polymorphism at the position; and e) selecting the first sequencing read and the second sequencing read for alignment based on the presence of the single polynucleotide polymorphism at the position in both the first 35
sequencing read and the second sequencing read. Some embodiments further comprise generating a candidate sequence by aligning the first sequencing read and the second sequencing read. In some embodiments, the presence of the single nucleotide polymorphism in the first sequencing 40
read is determined by comparison of the first sequencing read with another sequence. In some embodiments, the presence of the single nucleotide polymorphism in the first sequencing read is determined by comparison of the first sequencing read with a reference sequence. In some embodi- 45
ments, the sample is associated with a plurality of distinct barcodes. In some embodiments, the first sequencing read and the second sequencing read share a common barcode. Some embodiments further comprise receiving a third sequencing read, wherein the third sequencing read does not 50
comprise the common barcode, and filtering out the third sequencing read. Some embodiments further comprise: f) receiving a third sequencing read, wherein the third sequencing read comprises the position; g) determining that the third sequencing read does not have the single polynucleotide polymorphism at the position; and h) excluding the third sequencing read from alignment with the first sequencing read based on the absence of the single polynucleotide polymorphism at the position of the third sequencing read. Some embodiments further comprise estimating a likelihood 60
that the third sequencing read and the first sequencing read correspond to regions of a common chromosome. In some embodiments, the determining whether the third sequencing read and the first sequencing read correspond to regions of a common chromosome has an error rate of no greater than 1%. Some embodiments further comprise determining that 65
the first sequencing read corresponds to a first haploid in a

haploid pair, and that the third sequencing read corresponds to the second haploid in the haploid pair. In some embodiments, the determination that the first sequencing read corresponds to the first haploid in the haploid pair and that 5
the third sequencing read corresponds to the second haploid in the haploid pair comprises determining a level of homology between the first sequencing read and the third sequencing read. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. Some embodi- 10
ments further comprise outputting the selection of the first sequencing read and the second sequencing read for alignment. Some embodiments further comprise providing a therapeutic intervention to a subject based on a result of the method.

In some embodiments, the invention provides a method of analyzing sequencing data associated with a sample, the method comprising: a) receiving by a computer system a 20
first candidate sequence and a second candidate sequence, wherein the computer system comprises a processor; b) determining by the processor a pattern of single nucleotide polymorphisms or lack thereof within the first candidate sequence; c) determining a pattern of single nucleotide polymorphisms or lack thereof within the second candidate 25
sequence; and d) estimating a likelihood that the first candidate sequence and the second candidate sequence correspond to a common chromosome based on the pattern of single nucleotide polymorphisms or lack thereof within the first candidate sequence and the pattern of single nucleotide polymorphisms or lack thereof within the second candidate 30
sequence. In some embodiments, the estimation of the likelihood that the first candidate sequence and the second candidate sequence correspond to a common chromosome is based on a statistical analysis of the pattern of single nucleotide polymorphisms or lack thereof within the first 35
candidate sequence and the pattern of single nucleotide polymorphisms or lack thereof within the second candidate sequence. In some embodiments, the estimating the likelihood that the first candidate sequence and the second candidate sequence correspond to a common chromosome has an error rate of no greater than 1%. In some embodi- 40
ments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. Some embodiments further comprise out- 45
putting the selection of the first sequencing read and the second sequencing read for alignment. Some embodiments further comprise providing a therapeutic intervention to a subject based on a result of the method.

In some embodiments, the invention provides a method of analyzing sequencing data associated with a sample, the method comprising: a) receiving by a computer system a set 50
of sequencing reads, wherein all of the sequencing reads comprise a common position, wherein the computer system comprises a processor; b) determining by the processor that all of the sequencing reads in the set of sequencing reads correspond to a common haplotype; c) checking an identity of a nucleic acid residue at the common position in each 55
sequencing read in the set of sequencing reads; and e) estimating a likelihood that an error exists in at least one of the sequencing reads based on the checking. Some embodiments further comprise determining that each sequencing read shares a common nucleic acid residue at the common position. In some embodiments, the common nucleic acid residue at the common position is a single nucleotide polymorphism. Some embodiments further comprise deter-

mining that the identity of the nucleic acid residue at the common position in one of the sequencing reads is different from the identity of the nucleic acid residue at the common position in another one of the sequencing reads. In some embodiments, all of the sequencing reads in the set of sequencing reads share a common barcode. In some embodiments, the sample is associated with a plurality of distinct barcodes. Some embodiments further comprise receiving an additional sequencing read, wherein the additional sequencing read does not correspond to the common haplotype, and excluding the additional sequencing read from the set of sequencing reads. Some embodiments further comprise determining that the additional sequencing read corresponds to a second haplotype complementary to the common haplotype. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. Some embodiments further comprise outputting the estimated likelihood that an error exists in at least one of the sequencing reads.

In some embodiments, the invention provides a method of analyzing sequencing data associated with a sample, the method comprising: a) receiving by a computer system a first sequencing read and a second sequencing read, wherein the first sequencing read and the second sequencing read each comprise a common barcode, wherein the computer system comprises a processor; b) aligning the first sequencing read to a reference genome, wherein the reference genome comprises at least two reference chromosomes, and determining that the first sequencing read corresponds to a first reference chromosome in the reference genome; and c) aligning the second sequencing read to the reference genome and determining to which reference chromosome in the reference genome the second sequencing read corresponds, wherein the sample is associated with a plurality of distinct barcodes. In some embodiments, the aligning the second sequencing read to the reference genome determines that the second sequencing read corresponds to the first reference chromosome in the reference genome. In some embodiments, the aligning the second sequencing read to the reference genome determines that the second sequencing read corresponds to a second reference chromosome in the reference genome. Some embodiments further comprise estimating a likelihood that the sample contains a mutation from the reference genome. In some embodiments, the estimating the likelihood that the sample contains the mutation from the reference genome is based on the alignment of the first sequencing read to the reference genome and the alignment of the second sequencing read to the reference genome. Some embodiments further comprise determining a candidate site of the mutation in the reference genome. In some embodiments, the mutation is a translocation. Some embodiments further comprise: d) receiving by the computer system a plurality of further sequencing reads, wherein each further sequencing read comprises the common barcode; e) aligning each further sequencing read against the first reference chromosome in the reference genome; f) identifying a portion of the first reference chromosome that does not align to any of the further sequencing reads; and g) identifying the portion of the first reference chromosome that does not align to any of the further sequencing reads as the candidate breakpoint. Some embodiments further comprise determining a candidate breakpoint in the reference genome. In some embodiments, the candidate breakpoint is within 100 nucleotide residues of a sequencing read that aligns with the first reference chromosome. In some embodiments, the candidate breakpoint is within 50 nucleotide residues of a

sequencing read that aligns with the first reference chromosome. In some embodiments, the candidate breakpoint is within 10 nucleotide residues of a sequencing read that aligns with the first reference chromosome. Some embodiments further comprise determining which haplotype in a pair of haplotypes possesses the mutation. In some embodiments, the determining which haplotype in the pair of haplotypes possesses the mutation comprises identifying a common position among a subset of the further sequencing reads and identifying a common single nucleotide polymorphism at the common position of the further sequencing reads. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. Some embodiments further comprise outputting the determination of the reference chromosome in the reference genome to which the second sequencing read corresponds. Some embodiments further comprise providing a therapeutic intervention to a subject based on a result of the method.

In some embodiments, the invention provides a method of analyzing sequencing data associated with a sample, the method comprising: a) receiving by a computer system a set of sequencing reads, wherein each sequencing read independently comprises a barcode, wherein the computer system comprises a processor; b) sorting by the processor the sequencing reads of the set of sequencing reads into subsets, wherein each sequencing read of a first subset comprises a first common barcode, and each sequencing read of a second subset comprises a second common barcode, wherein the first common barcode and the second common barcode are not identical; and c) comparing the sequencing reads of the first subset to determine whether any sequencing reads of the first subset share a common subsequence in addition to the first barcode, wherein the sample is associated with a plurality of distinct barcodes. Some embodiments further comprise mutually aligning the sequencing reads of the first subset. Some embodiments further comprise excluding the sequencing reads of the second subset from the alignment. Some embodiments further comprise determining a candidate sequence based on the sequencing reads of the first subset. Some embodiments further comprise comparing the candidate sequence to a reference sequence. Some embodiments further comprise incorporating at least one of the sequencing reads of the second subset into the candidate sequence. Some embodiments further comprise determining a pattern of the first common barcode and the second common barcode based on the candidate sequence. Some embodiments further comprise scoring the candidate sequence based on the pattern of the first common barcode and the second common barcode. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. Some embodiments further comprise outputting a result of the comparing. Some embodiments further comprise providing a therapeutic intervention to a subject based on a result of analyzing the sequencing data.

In some embodiments, the invention provides a method of identifying sequencing errors in sequencing data associated with a sample, the method comprising: a) receiving by a computer system a first sequencing read and a second sequencing read, wherein the first sequencing read and the second sequencing read comprise a common barcode, wherein the first sequencing read and the second sequencing read share at least 90% homology, and wherein the computer system comprises a processor; b) comparing by the proces-

sor the first sequencing read and the second sequencing read to find at least one site at which the first sequencing read and the second sequencing read differ; and c) determining that the site in the second sequencing read is an error based on the comparing, wherein the sample is associated with a plurality of distinct barcodes. Some embodiments further comprise comparing the second sequencing read with a number of additional sequencing reads, wherein each of the additional sequencing reads comprises the common barcode and shares at least 90% homology with the second sequencing read. In some embodiments, a confidence level of the determining that the site in the second sequencing read is an error increases as the number of additional sequencing reads compared to the second sequencing read increases. Some embodiments further comprise fixing the error by changing a nucleic acid identity at the site in the second sequencing read. In some embodiments, the first sequencing read is longer than the second sequencing read. In some embodiments, the first sequencing read and the second sequencing read share at least 95% homology. In some embodiments, the first sequencing read and the second sequencing read share at least 99% homology. In some embodiments, the first sequencing read and the second sequencing read share at least 99.9% homology. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. Some embodiments further comprise outputting that the site in the second sequencing read is an error.

In some embodiments, the invention provides a method of analyzing sequencing data, the method comprising: a) receiving by a computer system a candidate sequence, wherein the candidate sequence is based on a set of sequencing reads, wherein each sequencing read is independently associated with one of at least two distinct barcodes, wherein the computer system comprises a processor; and b) analyzing by the processor the candidate sequence to identify a pattern of the barcodes associated with the candidate sequence. Some embodiments further comprise: c) selecting a sequencing read from the set of sequencing reads; and d) comparing the pattern of the barcodes associated with the candidate sequence and the barcode associated with the selected sequencing read. Some embodiments further comprise: e) relocating the selected sequencing based on the comparison of the pattern of the barcodes associated with the candidate sequence and the barcode associated with the selected sequencing read. In some embodiments, the candidate sequence corresponds to a reference chromosome, and the method further comprises relocating one of the sequencing reads to another position on the reference chromosome. In some embodiments, the candidate sequence corresponds to a reference chromosome, and the method further comprises relocating one of the sequencing reads to another reference chromosome. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. Some embodiments further comprise providing an output based on the analyzing.

In some embodiments, the invention provides a method of analyzing sequencing data, the method comprising: a) receiving by a computer system a first candidate sequence and a second candidate sequence, wherein the computer system comprises a processor; b) processing by the processor the first candidate sequence and the second candidate sequence to provide display data for both first candidate sequence and the second candidate sequence; and c) dis-

playing contemporaneously the first candidate sequence and the second candidate sequence. In some embodiments, the first candidate sequence is based on data obtained from a sequencing instrument. In some embodiments, the first candidate sequence is received before the sequencing assay is complete. In some embodiments, the display of the first candidate sequence is updated before the sequencing assay is complete. In some embodiments, the display of the second candidate sequence is updated contemporaneously with the update of the display of the first candidate sequence. In some embodiments, the second candidate sequence is based on data obtained from the sequencing instrument. In some embodiments, the second candidate sequence is based on data obtained from a different sequencing instrument. In some embodiments, the second candidate sequence is based on data obtained from a database. In some embodiments, the first candidate sequence and the second candidate sequence correspond to different chromosomes. In some embodiments, the different chromosomes are associated with a common sample. In some embodiments, the different chromosomes are each independently associated with a different sample. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. In some embodiments, the sequencing data is peptide sequencing data.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module configured to receive sequencing data associated with a sequencing assay while the sequencing assay is in progress; and ii) an alignment module configured to align sequencing data while the sequencing assay is in progress; b) receiving by the receiving module a set of sequencing reads associated with the sequencing assay while the sequencing assay is in progress; and c) performing an alignment by the alignment module of at least one received sequencing read with another sequence while the sequencing assay is in progress. In some embodiments, the sequencing data analysis system further comprises a database access module, wherein the method further comprises accessing a database to obtain the other sequence. In some embodiments, the sequencing data analysis system further comprises a reference sequence, wherein the other sequence aligned with the sequencing read is the reference sequence. In some embodiments, the sequencing data analysis system further comprises a barcode module, wherein the method further comprises identifying by the barcode module at least two sequencing reads in the set of sequencing reads that share a common barcode. In some embodiments, the sequencing data analysis system further comprises a candidate module, wherein the method further comprises building by the candidate module a candidate sequence based on the alignment. In some embodiments, the sequencing data analysis system further comprises a mutation module, wherein the method further comprises estimating by the mutation module a likelihood that the candidate sequence comprises a mutation. In some embodiments, the mutation is a single nucleotide polymorphism. In some embodiments, the mutation is a copy number variant. In some embodiments, the mutation is a translocation. In some embodiments, the sequencing data analysis system further com-

prises a haplotype module, wherein the method further comprises determining by the haplotype module to which haplotype in a pair of haplotypes one of sequencing reads of the set of sequencing reads corresponds. In some embodiments, the sequencing reads associated with the sequencing assay are received from an instrument conducting the sequencing assay. In some embodiments, the sequencing reads associated with the sequencing assay are received from a database. In some embodiments, the computer program product is in communication with a sequencing instrument. In some embodiments, the sequencing data analysis system further comprises a user information module, wherein the method further comprises obtaining by the user information module user information from the sequencing instrument. In some embodiments, the sequencing data analysis system further comprises a user information module, wherein the method further comprises obtaining by the user information module user information associated with a configuration of the sequencing instrument. In some embodiments, the computer program product is in communication with a plurality of sequencing instruments. In some embodiments, the sequencing data analysis system analyzes DNA sequencing data. In some embodiments, the sequencing data analysis system analyzes RNA sequencing data. In some embodiments, the sequencing data analysis system analyzes genomic sequencing data. In some embodiments, the sequencing data analysis system analyzes peptide sequencing data. In some embodiments, the sequencing data analysis system further comprises an output module, wherein the method further comprises outputting by the output module a result of the alignment.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module; ii) an alignment module; iii) a database access module; iv) a window module; and v) a barcode module; b) receiving by the receiving module a set of sequencing reads, wherein each sequencing read independently comprises a barcode; c) accessing by the database access module a database to obtain a reference sequence stored in the database; d) performing an alignment by the alignment module of each sequencing read of the set of sequencing reads with the reference sequence; e) selecting by the window module a window of the reference sequence; and f) counting by the barcode module a total number of distinct barcodes associated with the reference sequence within the window of the reference sequence based on the alignment. In some embodiments, the sequencing data analysis system further comprises a mutation module, wherein the method further comprises estimating by the mutation module a likelihood that a sample associated with the set of sequencing reads comprises a mutation based on the counting. In some embodiments, the sequencing data analysis system further comprises a haplotype module, wherein the method further comprises determining by the haplotype module which haplotype in a pair of haplotypes comprises the mutation. In some embodiments, the mutation is a copy number variant. In some embodiments, the method further comprises estimating by the mutation module the number of copies in the copy number variant. In some embodiments, the sequencing data analysis system analyzes DNA sequencing data. In some embodiments, the sequencing data analysis system analyzes RNA sequencing data. In

some embodiments, the sequencing data analysis system analyzes genomic sequencing data. In some embodiments, the sequencing data analysis system further comprises an output module, wherein the method further comprises outputting by the output module a result of the counting.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module; ii) an alignment module; iii) a database access module; and iv) a sorting module; b) receiving by the receiving module a set of sequencing reads, wherein each sequencing read shares a common position; c) accessing by the database access module a database to obtain a reference sequence stored in the database; d) performing an alignment by the alignment module of each sequencing read of the set of sequencing reads with the reference sequence; and e) sorting by the sorting module the sequencing reads of the set of sequencing reads into two subsets of sequencing reads, wherein a first subset of sequencing reads contains sequencing reads that match the reference sequence at the common position, and a second subset of sequencing reads contains sequencing reads that do not match the reference sequence at the common position. In some embodiments, each sequencing read of the second subset of sequencing reads possesses a single nucleotide polymorphism at the common position. In some embodiments, the sequencing data analysis system further comprises a haplotype module, wherein the method further comprises associating by the haplotype module the first subset of sequencing reads with a first haplotype of a pair of haplotypes, and the second subset of sequencing reads with the second haplotype of the pair of haplotypes. In some embodiments, the sequencing data analysis system further comprises a mutation module, wherein the method further comprises estimating by the mutation module a likelihood that either haplotype of the pair of haplotypes comprises a mutation based on a total number of sequencing reads in the first subset of sequencing reads and a total number of sequencing reads in the second subset of sequencing reads. In some embodiments, the sequencing data analysis system further comprises a barcode module, wherein the method further comprises identifying a common barcode within the a set of sequencing reads and excluding from the first subset of sequencing reads and the second subset of sequencing reads any sequencing read that does not comprise the common barcode. In some embodiments, the sequencing data analysis system analyzes DNA sequencing data. In some embodiments, the sequencing data analysis system analyzes RNA sequencing data. In some embodiments, the sequencing data analysis system analyzes genomic sequencing data. In some embodiments, the sequencing data analysis system further comprises an output module, wherein the method further comprises outputting by the output module a result of the sorting.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module; ii) an alignment module; iii) a database access module; and iv) a selection module; b) receiving by the receiving module a first sequencing read and a second

sequencing read, wherein the first sequencing read and the second sequencing read overlap at a common position; c) accessing by the database access module a database to obtain a reference sequence stored in the database; d) performing an alignment by the alignment module of the first sequencing read with the reference sequence, thereby determining a presence of a single nucleotide polymorphism at the common position in the first sequencing read; e) performing an alignment by the alignment module of the second sequencing read with the reference sequence, thereby determining a presence of the single nucleotide polymorphism at the common position in the second sequencing read; and f) selecting by the selection module the first sequencing read and the second sequencing read for mutual alignment based on the presence of the single nucleotide polymorphism at the common position in the first sequencing read and the second sequencing read. In some embodiments, the method further comprises aligning by the alignment module the first sequencing read and the second sequencing read. In some embodiments, the sequencing data analysis system further comprises a candidate module, wherein the method further comprises building by the candidate module a candidate sequence based on the alignment of the first sequencing read and the second sequencing read. In some embodiments, the sequencing data analysis system further comprises a barcode module, wherein the method further comprises determining by the barcode module that the first sequencing read and the second sequencing read share a common barcode. In some embodiments, the selecting by the selection module the first sequencing read and the second sequencing read for mutual alignment is further based on the first sequencing read and the second sequencing read sharing a common barcode. In some embodiments, the sequencing data analysis system further comprises a chromosome module, wherein the receiving module receives a third sequencing read, and wherein the method further comprises estimating by the chromosome module a likelihood that the third sequencing read and the first sequencing read correspond to regions of a common chromosome. In some embodiments, the sequencing data analysis system analyzes DNA sequencing data. In some embodiments, the sequencing data analysis system analyzes RNA sequencing data. In some embodiments, the sequencing data analysis system analyzes genomic sequencing data. In some embodiments, the sequencing data analysis system further comprises an output module, wherein the method further comprises outputting by the output module a result based on the selecting the first sequencing read and the second sequencing read for alignment.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module; ii) a pattern module; and iii) a chromosome module; b) receiving by the receiving module a first candidate sequence and a second candidate sequence; c) determining by the pattern module a pattern of single nucleotide polymorphisms or lack thereof within the first candidate sequence; d) determining by the pattern module a pattern of single nucleotide polymorphisms or lack thereof within the second candidate sequence; and e) estimating by the chromosome module a likelihood that the first candidate sequence and the second candidate sequence correspond to a common chromosome based on the pattern of single

nucleotide polymorphisms or lack thereof within the first candidate sequence and the pattern of single nucleotide polymorphisms or lack thereof within the second candidate sequence. In some embodiments, the sequencing data analysis system further comprises a statistical database access module, wherein the statistical database comprises statistical information associated with single nucleotide polymorphisms, wherein the estimating by the chromosome module the likelihood that the first candidate sequence and the second candidate sequence correspond to the common chromosome comprises comparing, by the chromosome module, the pattern of single nucleotide polymorphisms or lack thereof within the first candidate sequence and the pattern of single nucleotide polymorphisms or lack thereof within the second candidate sequence to information in the statistical database via the statistical database access module. In some embodiments, the sequencing data analysis system analyzes DNA sequencing data. In some embodiments, the sequencing data analysis system analyzes RNA sequencing data. In some embodiments, the sequencing data analysis system analyzes genomic sequencing data. In some embodiments, the sequencing data analysis system further comprises an output module, wherein the method further comprises outputting by the output module an estimated likelihood that the first candidate sequence and the second candidate sequence correspond to a common chromosome.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module; ii) a haplotype module; iii) a nucleotide-recognition module; and iv) an error module; b) receiving by the receiving module a set of sequencing reads, wherein all of the sequencing reads comprise a common position; c) determining by the haplotype module that all of the sequencing reads in the set of sequencing reads correspond to a common haplotype; d) recognizing by the nucleotide-recognition module a nucleic acid residue at the common position in each of the sequencing reads in the set of sequencing reads independently; and e) estimating by the error module a likelihood that an error exists in at least one of the sequencing reads in the set of sequencing reads based on the identifying. In some embodiments, the sequencing data analysis system further comprises a reference sequence and an alignment module, wherein the method further comprises determining by the alignment module that at least one sequencing read in the set of sequencing reads has a single nucleotide polymorphism at the common position based on an alignment of the sequencing read with the reference sequence. In some embodiments, the sequencing data analysis system further comprises a barcode module, wherein the method further comprises determining by the barcode module that the sequencing reads in the set of sequencing reads share a common barcode. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. In some embodiments, the sequencing data analysis system further comprises a correction module, wherein the method further comprises correcting the error by the correction module. In some embodiments, the correcting the error by the correction module comprises replacing a nucleic acid residue in one of the sequencing reads with an alternative nucleic acid residue. In some embodiments, the

sequencing data analysis system further comprises an output module, wherein the method further comprises outputting by the output module an estimated likelihood that an error exists in at least one of the sequencing reads.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module; ii) an alignment module; iii) a database access module; and iv) a mutation module; b) receiving by the receiving module a first sequencing read and a second sequencing read; c) accessing by the database access module a database to obtain a reference genome stored in the database, wherein the reference genome comprises at least two reference chromosomes; d) performing an alignment by the alignment module of the first sequencing read with the reference genome, thereby determining that the first sequencing read corresponds to a first reference chromosome in the reference genome; e) performing an alignment by the alignment module of the second sequencing read with the reference genome, thereby determining that the second sequencing read corresponds to a second reference chromosome in the reference genome; and f) estimating by the mutation module a likelihood that a mutation exists in a sample associated with the first sequencing read and the second sequencing read based on the alignments of the first sequencing read and the second sequencing read with the reference genome. In some embodiments, the method further comprises determining by the mutation module a candidate site of the mutation based on the alignments of the first sequencing read and the second sequencing read with the reference genome. In some embodiments, the mutation module is configured to identify a translocation, and wherein the estimating by the mutation module a likelihood that a mutation exists in the sample associated with the first sequencing read and the second sequencing read comprises estimating a likelihood that a translocation exists in the sample associated with the first sequencing read and the second sequencing read. In some embodiments, the mutation module is configured to identify a candidate breakpoint associated with the translocation, wherein the method further comprises identifying by the mutation module a candidate breakpoint associated with the translocation based on the alignments of the first sequencing read and the second sequencing read with the reference genome. In some embodiments, the sequencing data analysis system further comprises a barcode module, and the method further comprises: g) receiving by the receiving module a plurality of further sequencing reads; h) determining by the barcode module that the first sequencing read and the further sequencing reads comprise a common barcode; i) aligning by the alignment module each further sequencing read against the first reference chromosome in the reference genome; and j) identifying by the alignment module a portion of the first reference chromosome that does not align to any of the further sequencing reads. In some embodiments, the sequencing data analysis system further comprises a haplotype module, wherein the method further comprises determining by the haplotype module which haplotype in a pair of haplotypes has the mutation. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. In some embodiments, the

sequencing data analysis system further comprises an output module, wherein the method further comprises outputting by the output module an estimated likelihood that the mutation exists in the sample associated with the first sequencing read and the second sequencing.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module; ii) a barcode module; and iii) an alignment module; b) receiving by the receiving module a first set of sequencing reads and a second set of sequencing reads; c) determining by the barcode module that the sequencing reads of the first set of sequencing reads share a first common barcode; d) determining by the barcode module that the sequencing reads of the second set of sequencing reads share a second common barcode, wherein the first common barcode and the second common barcode are not identical; and e) performing an alignment by the alignment module of one sequencing read of the first set of sequencing reads with one sequencing read of the second set of sequencing reads. In some embodiments, the sequencing data analysis system further comprises a candidate module, wherein the method further comprises determining a candidate sequence by the candidate module based on the alignment of one sequencing read of the first set of sequencing reads with one sequencing read of the second set of sequencing reads. In some embodiments, the sequencing data analysis system further comprises a pattern module, wherein the method further comprises determining a pattern by the pattern module of the first common barcode and the second common barcode based on the candidate sequence. In some embodiments, the sequencing data analysis system further comprises a scoring module, wherein the method further comprises scoring by the scoring module the candidate sequence based on the pattern of the first common barcode and the second common barcode. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. In some embodiments, the sequencing data analysis system further comprises an output module, wherein the method further comprises outputting by the output module the alignment of one sequencing read of the first set of sequencing reads with one sequencing read of the second set of sequencing reads.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module; ii) a barcode module; and iii) a pattern module; b) receiving by the receiving module a candidate sequence; c) determining by the barcode module that the candidate sequence is associated with a plurality of distinct barcodes; and d) determining by the pattern module a pattern of the barcodes associated with the sequencing reads. In some embodiments, the candidate sequence is based on a set of sequencing reads, wherein the method further comprises determining by the barcode module which barcode of the plurality of distinct barcodes is independently associated with each sequencing read in the set of sequencing reads. In

some embodiments, the sequencing data analysis system further comprises a selection module, wherein the method further comprises selecting by the selection module one of the sequencing reads, and comparing by the pattern module the pattern of barcodes associated with the candidate sequence and the barcode associated with the selected sequencing read. In some embodiments, the sequencing data analysis system further comprises a relocation module, wherein the method further comprises relocating by the relocation module the selected sequencing read based on the comparison of the pattern of barcodes associated with the candidate sequence and the barcode associated with the selected sequencing read. In some embodiments, the candidate sequence corresponds to a candidate chromosome, and the selected sequencing read is relocated to another position on the candidate chromosome. In some embodiments, the candidate sequence corresponds to a candidate chromosome, and the selected sequencing read is relocated to another candidate chromosome. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the sequencing data is genomic sequencing data. In some embodiments, the sequencing data analysis system further comprises an output module, wherein the method further comprises outputting by the output module an output based on the pattern of the barcodes associated with the sequencing reads.

In some embodiments, the invention provides a computer program product comprising a computer-readable medium having computer-executable code encoded therein, the computer-executable code adapted to be executed to implement a method of analyzing sequencing data, the method comprising: a) providing a sequencing data analysis system, wherein the sequencing data analysis system comprises: i) a receiving module; ii) a first display module; and iii) a second display module; b) receiving by the receiving module a first candidate sequence and a second candidate sequence; c) displaying by the first display module the first candidate sequence; and d) displaying by the second display module the second candidate sequence contemporaneously with the displaying by the first display module of the first candidate sequence. In some embodiments, the first candidate sequence is received before a sequencing assay that generated the first candidate sequence is complete. In some embodiments, the sequencing data analysis system further comprises an update module, wherein the method further comprises updating by the update module the display of the first candidate sequence before a sequencing assay that generated the first candidate sequence is complete. In some embodiments, the method further comprises updating by the update module the display of the second candidate sequence contemporaneously with the update of the display of the first candidate sequence. In some embodiments, the sequencing data analysis system is in communication with a sequencing instrument, wherein the first candidate sequence is based on sequencing data received from the sequencing instrument. In some embodiments, the second candidate sequence is based on sequencing data received from the sequencing instrument by the receiving module. In some embodiments, the second candidate sequence is based on sequencing data received from a database by the receiving module. In some embodiments, the sequencing data is DNA sequencing data. In some embodiments, the sequencing data is RNA sequencing data. In some embodiments, the

sequencing data is genomic sequencing data. In some embodiments, the sequencing data is peptide sequencing data.

What is claimed is:

1. A sequencing analysis system, comprising:

one or more computer processors in communication with a sequencer, said sequencer configured to sequence a plurality of nucleic acid molecules and generate a set of partial sequencing reads comprising a partial sequencing read comprising a subsequence, wherein said subsequence comprises a sequencing error, wherein said one or more computer processors are individually or collectively configured to:

(i) while said sequencer is sequencing said plurality of nucleic acid molecules, process said partial sequencing read based at least in part on a frequency of said subsequence among at least a portion of said set of partial sequencing reads to thereby correct said sequencing error, and

(ii) while said sequencer is sequencing said plurality of nucleic acid molecules, generate, for display, an indication of said sequencing error in said subsequence.

2. The system of claim 1, wherein said one or more computer processors are individually or collectively configured to, while said sequencer is sequencing said plurality of nucleic acid molecules, receive user input through an interface in communication with said one or more computer processors.

3. The system of claim 1, wherein said subsequence comprises at least a portion of a barcode sequence.

4. The system of claim 1, wherein said one or more computer processors are individually or collectively configured to process said partial sequencing read and an additional partial sequencing read comprising said subsequence to identify said sequencing error or generate a consensus sequence.

5. The system of claim 1, wherein said one or more computer processors are in communication with a database, and wherein said one or more computer processors are individually or collectively configured to store said set of partial sequencing reads in said database.

6. The system of claim 1, wherein said one or more computer processors are individually or collectively configured to receive said set of partial sequencing reads from a data stream while said sequencer is sequencing said plurality of nucleic acid molecules.

7. The system of claim 1, wherein said one or more computer processors are in communication with said sequencer over a cloud infrastructure.

8. A sequencing analysis system, comprising:

one or more computer processors in communication with a sequencer, said sequencer configured to sequence a plurality of nucleic acid molecules and generate a set of partial sequencing reads, wherein a partial sequencing read of said set of partial sequencing reads comprises at least a portion of a barcode sequence, wherein said one or more computer processors are individually or collectively configured to:

while said sequencer is sequencing said plurality of nucleic acid molecules, receive, from said sequencer, said set of partial sequencing reads; and

while said sequencer is sequencing said plurality of nucleic acid molecules, analyze said partial sequencing read of said set of partial sequencing reads based at least in part on said at least said portion of said barcode sequence.

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9. The system of claim 8, wherein said one or more computer processors are individually or collectively configured to process (i) said partial sequencing read comprising said portion of said barcode sequence and (ii) an additional partial sequencing read of said set of partial sequencing reads comprising an additional portion of said barcode sequence to assemble said barcode sequence.

10. The system of claim 8, wherein said one or more computer processors are individually or collectively configured to assign a subset of said set of partial sequencing reads to a group of a plurality of groups based on said at least said portion of said barcode sequence.

11. The system of claim 8, wherein said one or more computer processors are individually or collectively configured to assemble said partial sequencing read with at least one other read of said set of partial sequencing reads.

12. The system of claim 8, wherein said set of partial sequencing reads comprises a second partial sequencing read, and wherein said one or more computer processors are individually or collectively configured to process said partial sequencing read and said second partial sequencing read to determine whether said partial sequencing read and said second partial sequencing read share a common subsequence.

13. The system of claim 12, wherein said one or more computer processors are individually or collectively configured to identify a genetic variant in said partial sequencing read or said second partial sequencing read.

14. The system of claim 12, wherein said one or more computer processors are individually or collectively configured to determine whether said partial sequencing read and said second partial sequencing read correspond to a common biomolecule.

15. The system of claim 8, wherein said one or more computer processors are individually or collectively configured to process said partial sequencing read with a reference sequence.

16. The system of claim 15, wherein said one or more computer processors are individually or collectively configured to process said partial sequencing read with said reference sequence to identify a mutation in said partial sequencing read.

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17. The system of claim 8, wherein said one or more computer processors are individually or collectively configured to determine a candidate sequence corresponding to a chromosome, wherein said candidate sequence incorporates a portion of said partial sequencing read.

18. The system of claim 17, wherein said one or more computer processors are individually or collectively configured to determine said candidate sequence at a rate that increases as a number of partial sequencing reads received by said one or more computer processors increases.

19. The system of claim 8, wherein said one or more computer processors are individually or collectively configured to determine a haplotype in a pair of haplotypes to which said partial sequencing read corresponds.

20. The system of claim 8, wherein said one or more computer processors are individually or collectively configured to receive said set of partial sequencing reads from a data stream while said sequencer is sequencing said plurality of nucleic acid molecules.

21. A sequencing analysis system, comprising:
one or more computer processors in communication with a sequencer over a cloud, said sequencer configured to sequence a nucleic acid molecule to generate a partial sequencing read,
wherein said one or more computer processors are individually or collectively configured to, while said sequencer is sequencing said nucleic acid molecule, (i) receive said partial sequencing read from said sequencer over said cloud, and (ii) process said partial sequencing read to yield a result.

22. The system of claim 21, wherein said one or more computer processors are in communication with a database, and wherein said one or more computer processors are individually or collectively configured to store said partial sequencing read in said database.

23. The system of claim 21, wherein said one or more computer processors are individually or collectively configured to receive said partial sequencing read from a data stream while said sequencer is sequencing said nucleic acid molecule.

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